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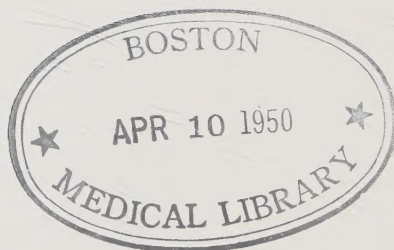
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THE OSSICLES OF THE SEMILUNAR CARTILAGES OF RODENTS¹

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SIX FIGURES

INTRODUCTION

Watson-Jones ('34) reported two instances of ossification of the semilunar cartilages of man. In each case there was an ossicle completely surrounded by normal cartilage with no accompanying evidence of trauma or inflammation to explain its development. Harris ('34), in a special effort to explain the presence of that bone, examined the knee joints of rats and stated, "the relatively stable condition of the meniscus in the adult rat presents a disc in which a core of bone and marrow is surrounded by a narrow rim of calcified cartilage and a wider rim of cartilage which becomes fibrous toward the free surface." He presented photomicrographs to demonstrate that the bone was absent at birth, was constantly present in the adult and that its development was first indicated by changes in the cartilage at about 6 weeks of age.

Green ('35) described the skeleton of the rat and stated that the "semilunar cartilages of the knee joint are ossified." Pearson and Davin ('20), in two papers on the sesamoids of the knee joint, described the presence of "lunulae" in the semilunar cartilages of many animals. They described

¹ Aided by a Fellowship from the National Foundation for Infantile Paralysis.

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"lunulae" as small ossicles within the cartilage, which occurred in 6 positions: anterolateral, anterosagittal and anteromesial, both anteriorly and posteriorly. In their study of rodents they found the anteromesial lunulae constantly, and occasionally they found one in a posterior position. Their work was based on gross dissection of single specimens or inspection of mounted specimens. No microscopic studies were made.

Strong ('25), in his study of the ossification centers of the rat, did not mention ossification of the semilunar cartilage. Dawson ('25) reported on the order of epiphyseal closure in the rat. He did not discuss ossification of menisci but in one of his plates noted the presence of bone within a meniscus.

It is apparent that there is a difference of opinion as to the extent of ossification in the menisci of rodents. The purpose of this paper is to present evidence supporting Pearson and Davin and to discuss briefly the significance of the ossification.

MATERIALS AND METHODS

The knee joints of the hamster, mouse, guinea pig and rat were examined grossly, with the dissecting microscope and by microscopic section. Serial sections 10μ thick were made; every 10th section was mounted and stained with either Hematoxylin and Eosin or with the Masson tri-chrome stain. Joints were sectioned in both the sagittal and frontal plane. Rat knee joints were examined from birth up to a known age of over two years. Both knee joints of approximately 40 animals were studied. In addition to the joints sectioned serially, those from animals sacrificed in other experiments were examined.

RESULTS

With the aid of the dissection microscope, roughly round or oval areas of discoloration were visible in the hyaline cartilage of the menisci. These areas in fresh specimens were yellow to tan. The cartilage covering the area was slightly

elevated above the remainder of the meniscus. Such nodules were found in the anterior portion of every meniscus examined from the adult hamster, mouse and rat. In two rats, weighing 350 gm each, but of unknown age, and in 6 rats whose known age was over two years, there were additional nodules in the posterior portions of the lateral menisci. Figure 1 is a drawing made from one of those rat knee joints.

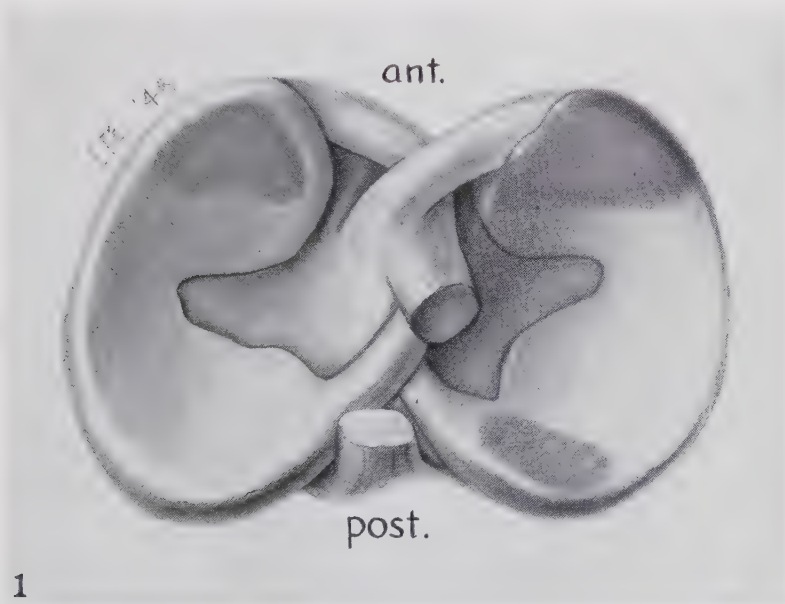


Fig. 1 Drawing of the proximal articular surface of the tibia of the rat, with menisci intact. The shaded areas represent the intra-meniscal ossicles.

The shape and position of the ossicles, as illustrated, is typical of all the joints examined, except for the posterior one which was present only in older rats. In none of the joints examined was there any structural change which could be attributed to trauma or inflammation.

Microscopic study of the stained sections revealed that the nodules were composed of bone. Each nodule consisted of an outer layer of lamellar bone, within which were trabeculae and marrow. The nodules were entirely surrounded by

hyaline cartilage. In no case was there any other indication of ossification or calcification. There was no microscopic evidence of trauma, inflammation, or degenerative joint changes, such as fibrillation of cartilage or excess proliferation of bone. Figure 2 demonstrates the anterior position of the nodule of bone and the absence of bone in the posterior portion of the same meniscus. Figures 3, 4, 5, and 6 illustrate the similarity of bone formation in the hamster, mouse, rat and guinea pig.

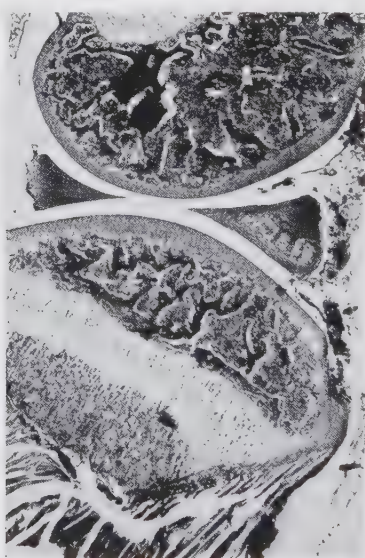


Fig. 2 Sagittal section through the knee joint of the mouse to demonstrate the presence of bone in the anterior portion of the meniscus and the absence of bone in the posterior portion. $\times 15$ Masson Tri-Chrome.

DISCUSSION

The above findings appeared to substantiate the statements of Pearson and Davin ('20) on the presence of "lunulae" in rodents. Since they were concerned with the gross, comparative anatomy of the sesamoids of the knee joint, their illustrations did not conclusively demonstrate the presence of bone in the menisci. They did find bone, however, and re-

ported its occurrence in many animals. They indicated that whereas the "lunulae" were constantly present in lower forms, they became increasingly rare, the higher the order of animal. For man, they presented radiographs in which there were opaque nodules which might be interpreted as being in the menisci. Those joints were not opened, however, and the films were not conclusive.

The existence of both calcification and ossification in the menisci of man has been reported. Weaver ('42) reviewed the literature and pointed to the existence of primary and secondary types of both calcification and ossification. The secondary type followed trauma and primary types were unexplained. Watson-Jones ('34) stated that the finding of ossicles within the cartilage of the menisci of man, in the absence of evidence of trauma, inflammation or degeneration was explainable as a true example of heterotopic bone formation. Harris ('34) felt that his findings in the rat indicated that ossification was a degenerative change, dependent on the growth of the meniscus beyond its source of nutrition. That lack of nutrition was first felt at the center of the cartilage and, consequently, throughout the core of the cartilage, the cells became hypertrophied, died, calcified and were finally invaded by blood vessels from the periphery.

It is felt that there are objections to the explanations of both the above authors. There is nothing in the rat knee joint to support the contention that a core of bone forms as a degenerative process. It is felt that in the normal rat meniscus, there is no core of bone. There are constantly present ossicles which develop within the cartilage, and those changes seen in the cartilage cells are the changes which usually accompany the formation of bone. It is difficult to consider a process a degenerative one when it is constantly present in otherwise healthy young adult animals.

Heterotopic bone formation is considered to be the formation of bone where bone is ordinarily not formed. It results from the fortuitous presence of the combination of factors necessary for the production of bone, i.e., an ossifiable

medium, a source of calcium, a blood supply. It has been pointed out that in the rat, the ossicle is constantly present and, by definition, cannot represent heterotopic bone formation. In the cases reported by Watson-Jones, there was no evidence of any change in local conditions such as might lead to bone formation. On the other hand, Pearson and Davin ('20) have shown that the higher the order, the lower the incidence of "lunulae." It seems more probable that the occasional intra-meniscal ossicle in man, in the absence of disease or degenerative process, is a vestigial structure.

SUMMARY AND CONCLUSIONS

1. Gross and microscopic studies of the menisci of rodents were reported.
2. Ossicles were constantly present in the anterior portions of the menisci and frequently in the posterior portions as well.
3. The ossicles were completely surrounded by cartilage and were found in joints showing no evidence of disease.
4. Nothing was found to indicate that the entire semilunar cartilage in the rat was completely ossified or that there was a core of bone running throughout the cartilage.
5. Reports on the ossification of the semilunar cartilages in man were reviewed and the reason for such ossification discussed.
6. It is felt that a completely intra-meniscal ossicle in man may be a vestigial structure but that it is of normal occurrence in rodents.

ACKNOWLEDGMENTS

The author wishes to express thanks to Miss Evelyn Erikson, Department of Medical Illustration, Wayne University, for her very accurate drawing and to Dr. Ernest Gardner, Department of Anatomy, Wayne University, for the photomicrographs.

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PLATE I

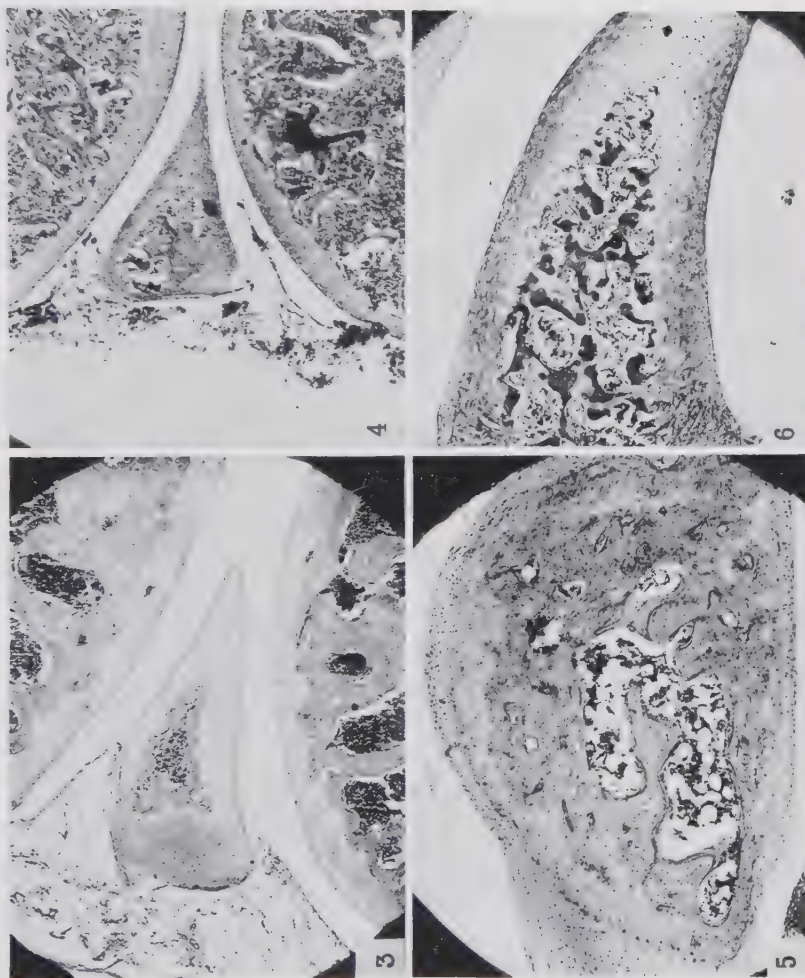
EXPLANATION OF FIGURES

Detail view through portions of menisci containing bone.

- 3 Hamster. $\times 60$ H and E.
- 4 Mouse. $\times 60$ Masson Tri-chrome.
- 5 Rat. $\times 60$ H and E.
- 6 Guinea pig. $\times 60$ Masson Tri-Chrome.

SEMILUNAR CARTILAGES OF RODENTS
HERBERT E. PEDERSEN

PLATE 1



A SIMPLE MACROSCOPIC STAINING AND MOUNTING PROCEDURE FOR WET SECTIONS FROM CADAVER BRAINS

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ONE FIGURE

INTRODUCTION

Wet sections of cadaver brains, cut coronally and horizontally, are always useful in the laboratory teaching of neuroanatomy. Students notice, however, that one series may show less differentiation between the white and the gray matter than another series. It is our aim to describe a method by which the white and gray matter of many cadaver brains can be differentially stained in a brilliant, permanent, yet simple fashion (fig. 1).

Students also show a more or less understandable reluctance to work with wet specimens fished out of a weak solution of fixative. Their reluctance can be overcome if the preparations are pleasant and convenient to handle and they will refer to the gross material more readily. Castolite or Ward's Bio-plastic can be used for this purpose also but these plastics are expensive and somewhat difficult to apply. A simpler, yet effective method for mounting the sections in plastics is suggested and described.

METHODS

Section preparation

Since not all cadaver brains are in good condition the first step is to determine which ones will be suitable for the stain.

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This depends on several factors: (1) the promptness and thoroughness of the embalmer's vascular perfusion with the fixative; (2) the nature of that fixative; (3) whether or not a fixative is directly injected into the cranial cavity; (4) the lapse of time after embalming; and (5) the treatment of the

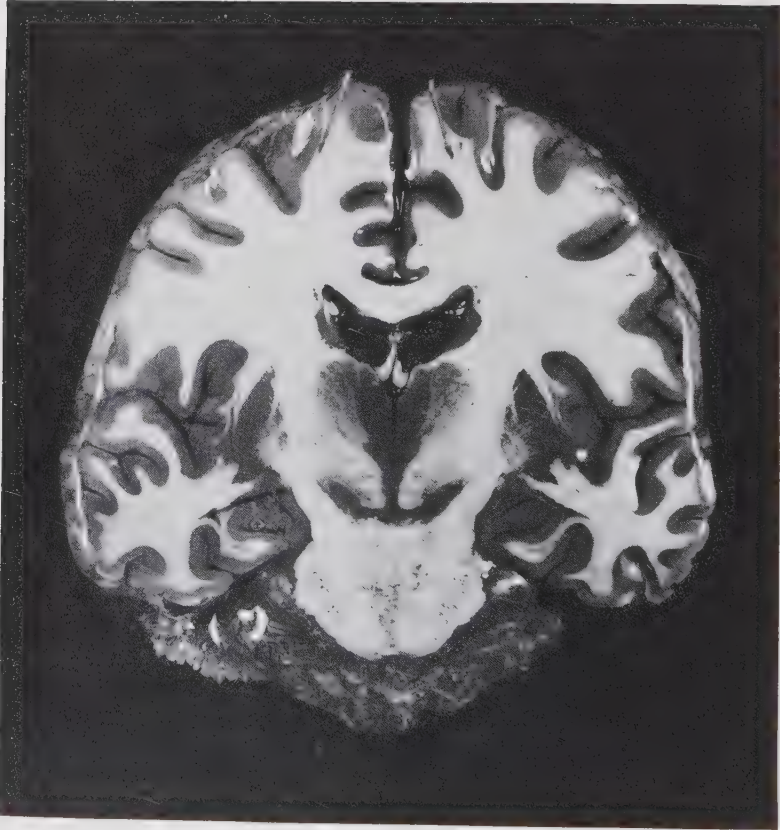


Fig. 1 A coronal section of a cadaver brain stained with Prussian blue and coated with butyl methacrylate polymer 4.

brain after its removal from the cadaver. Other factors might be listed but this would serve no useful purpose. In order to select the brains we have adopted the procedure of taking a 20 mm slice from the frontal pole and giving it a trial stain. If the results are unsatisfactory the brain can still

be used for unstained sections or as a whole or half brain. If the specimen stains well the brain is then stripped of its pia and arachnoid. This step is optional, but it does make the edges of the sections look neater and stain more uniformly. The brain is then sliced in the usual manner to produce as few knife marks as possible. We are fortunate to have an electric meat slicer for the purpose. Hand cutting does just as well, although even sections cannot readily be cut as thin as 25 mm which we do with the electric slicer. At less than 25 mm they do not hold their shape well.

A needle with heavy thread is inserted in the middle of the dorsal edge of the specimen close to the midline, and is pushed ventrally until it emerges in the ventricle. The needle is then reinserted through the medial wall of the ventricle until it comes out into the ventricle of the opposite side. It is then thrust through the roof of the ventricle to emerge finally from the dorsal edge of the section. The protruding pieces of thread are tied together close to the specimen. The thread is cut leaving an 18 inch free end. From this moment on the sections are lifted and handled by the thread. It is important not to handle or roughen the cut surfaces because, as LeMasurier ('35) has warned, it is very easy to leave finger prints that will show up in the stain. The sections are placed in tap water until it is convenient to stain them. If they are to be kept for more than a day they should be stored in 10% formalin.

Staining

The following staining procedure is modified from LeMasurier ('35).

Mulligan's solution ('31)

Phenol crystals	4.0	gm
(or 89% phenol solution — 4.5 ml)		
Copper sulphate	0.5	gm
Hydrochloric acid	0.125	ml
Distilled water	100.0	ml

1. Sections are placed for two minutes in hot (60° to 65°C.) Mulligan's solution sufficient in amount to cover the section by an inch or two of fluid.

2. Rinse in running tap water one minute.

3. Immerse in 1% ferric chloride one to three minutes until the gray is well differentiated.

4. Rinse in running tap water one minute.

5. Immerse in 1% potassium ferrocyanide until the gray matter is a brilliant blue.

6. Rinse in running tap water one minute.

7. If the blue is not intense enough reimmerse in 1% ferric chloride for a minute or less.

8. Rinse in running tap water for a minute.

9. Immerse in 10% formalin to which is added enough HCl to make 2% HCl for at least 24 hours. This step turns the white matter a clear white and removes all brownish stains which originally were in the tissue. The acidity is necessary to maintain the Prussian blue reaction. Older studies point out the importance of refixing the sections in formalin if the stain is to be preserved. One wonders if this is not to make the sections acid which will maintain the adsorption of the complex iron salt of the Prussian blue reaction.

10. If for any reason one should want to destain the sections after the Prussian blue reaction it may be done by placing the sections into 10% NH_4OH until all the blue color is removed. The sections will then be a dirty brown but this latter is easily removed by placing the sections overnight in a 2% hydrochloric acid solution. They may then be restained by starting with step 1.

The sections are now permanently and brilliantly stained. Blue discolorations in the white matter will be in inverse proportion to the original quality of the brain. Many of our cadaver brains stain well without any blue in the white matter. All of the gray matter will not take the same intensity of blue as Blair, Davies and McClelland ('32) have pointed out. For example, globus pallidus stains more lightly

that putamen. The red nucleus is outlined but its interior does not stain well. These and other departures from the expected will be noted (fig. 1).

Since the stain is confined to the surfaces of the sections they should not be scratched as this will reveal the unstained interior of the preparations.

Nigrosin as used by Jones ('49, mimeograph) does not give as brilliant contrast as the Prussian blue reaction reported here. Prussian blue will not fade if we are to believe Sinke ('26), Mainland ('28) and LeMasurier ('35). We have seen no signs of fading in 6 months even in bright sunlight.

Mounting

The stained sections (or unstained sections, whole brains etc.) are now dipped in plastic as described by Peck and Gray ('48). First they are surface dried by blotting. This is not a critical step since any extra water will sink to the bottom of the dipping solution as soon as the sections are immersed. However, it seems best to keep this excess fluid to the minimum. They are then hung by their thread and dipped repeatedly in a xylol solution of DuPont's butyl methacrylate polymer 4. Two parts of xylol by volume (milliliters) to one part of plastic weight (grams) make about the right consistency for dipping. On the first dip a camel hair brush is used to free air bubbles from the surface of the brain. Fifteen minutes later (if evaporating conditions are good) the brain may be dipped again. It should be dipped about 20 times in all but it may be done at one's convenience. It is important not to leave them over the weekend without at least 6 coats. After 15 to 20 coats the sections are water tight and may be left for further hardening. After a few days they can be handled but should not be laid on top of one another for at least two weeks. At this time the sections have the pleasant feel of smooth plastic and provide perfect visibility of the structures underneath. The plastic is pliable but the specimen should not be bent too freely since it might rupture the

methacrylate coat. Reasonable handling has no effect. As pointed out by Peck and Gray ('48) it can be used for many types of wet specimens.

The method is less liable to error than the one using Ward's Bio-plastic or Castolite. The results (fig. 1) are not superior to the beautiful Castolite preparations of Kampmeier and Haviland ('48), to the Bio-plastic mounts of Louise Jones or to some Castolite preparations in this laboratory. However, the "fool-proof" nature of the methacrylate dipping method found warm acceptance in our hands. We estimate the cost to be about one-fifth as much per brain section as with Castolite or Ward's Bio-plastic.

It has been our experience that the sharp and colorful differentiation of white and gray matter plus the pleasurable dry feel of the specimens make the study of brain sections, if not a pleasure, at least, less of an irritation to both student and teacher.

SUMMARY

The macroscopic staining and plastic embedding of wet sections of cadaver brains are described under three headings.

1. *Section preparation* consists of selecting and preparing the brain sections for staining.

2. *Staining* is a matter of obtaining the Prussian blue reaction in gray matter, while turning the "white" matter to a clean white.

3. *Mounting* consists of simple, repeated coating with a xylol solution of butyl methacrylate polymer 4 until a sufficient layer of plastic covers the preparation to insure its protection from handling and dehydration.

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THE HISTOLOGY OF THE ADRENAL GLAND IN THE ALLIGATOR LIZARD, *GERRHONOTUS* *MULTICARINATUS*

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TEN FIGURES

INTRODUCTION

The histology of the adrenal glands of lizards has received no attention except for the brief descriptions by Krause ('22) and Radu ('34). This study was made in an effort to contribute something further on the structural details of reptilian adrenals.

MATERIALS AND METHODS

Adrenal glands were studied from both sexes of 40 mature North American alligator lizards, including *Gerrhonotus multicarinatus scincicauda* (Skilton), the Oregon alligator lizard; *G. multicarinatus multicarinatus* (Blainville), the red-backed alligator lizard; and *G. multicarinatus webbi* Baird, the San Diego alligator lizard. These represent all the subspecies of *G. multicarinatus* recorded for North America (Smith, '46).

The lizards were anesthetized by intra-peritoneal injections of 5 mg of sodium pentobarbital (1:100 solution in 0.9% saline) to facilitate removal of the adrenals from the living animals.

The most satisfactory general fixative was found to be Zenker's solution in which 20% neutral formol was substituted for the acetic acid. This solution both preserved interrenal cell lipoids and gave the chromaffin reaction (Henle, 1865).

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Optimum fixation time with this fixative was found to be two and one-half hours; longer periods hardened the tissues excessively.

Although the da Fano method was useful in locating the Golgi bodies, it failed to demonstrate any of the finer details of structure. Attempts to impregnate the Golgi apparatus with osmium tetroxide were unsuccessful.

Mitochondria were satisfactorily fixed with Zenker-formol-osmic acid. The staining of mitochondria by the Altman-Cowdry anilin-acid fuchsin-methyl green method (Guyer, '36) was successful only after a potassium dichromate mordant (4 hours minimum).

Nerve fibers in both chromaffin and interrenal tissues were clearly stained by the Pearson-O'Neill ('46) silver-gelatine method following Bouin fixation. Methylene blue in 70% alcohol was used as a counterstain.

Dioxane was used as a rapid dehydrating agent after fixation, since it does not shrink and harden tissues as does the slower graded-alcohol method. All tissues were embedded in paraffin for sectioning. Haupt's adhesive (Johausen, '40) was used in preference to Mayer's albumen to fix sections to the slides where prolonged staining methods were employed, such as the Foot-Menard technique for reticular connective tissue.

Nuclear and cytoplasmic stains included iron hematoxylin, Harris' hematoxylin-eosin, Van Giesen's, Harris' hematoxylin-azure II-eosin (Lillie, '48) and methylene blue-eosin. Three connective tissue staining methods were employed: Mallory's triple for collagenous fibers, Foot-Menard's for reticular fibers (Lillie, '48) and MacCallum-Verhoff's for elastic fibers (Krajian, '40).

Wiesel's method (Krajian, '40) in which alcoholic methylene blue was substituted for toluidine blue was satisfactory for differentiating chromaffin cells. Adrenals fixed with Zenker-formol-osmic acid and counterstained with iron hematoxylin clearly demonstrated the osmiophilic secretion droplets of the interrenal cells.

OBSERVATIONS

Anatomical relationships

Both adrenal glands of *Gerrhonotus* are located near the cephalic poles of the gonads (fig. 2). While the right adrenal lies dorso-medially to the gonad with its anterior half projecting forward beyond the gonad almost to the caudal tip of the right liver lobe, the left adrenal lies at a level slightly caudal to that of the right, with its anterior two-thirds projecting free beyond the gonad.

In shape the adrenals appear ellipsoid (longitudinally disposed) but roughly rectangular in cross-section (fig. 2).

The gland surfaces are smoothly rounded with the cephalic and caudal poles narrowing to a somewhat pointed form.

In size the glands varied from 1.0 to 2.5 mm in length and from 0.5 to 1.5 mm in breadth (35 specimens); in 5 others, they were so small as to be barely visible to the unaided eye.

The large vein draining the adrenal lies on the ventral surface of the caudal portion of the gland (fig. 2). The spermatic duct in the male and the oviduct in the female is found in intimate contact with the dorsal surface of the adrenal along its entire length.

The right adrenal is suspended in the mesorchium of the male (in the mesovarium of the female) and slightly lateral to the midline. Both mesorchium and mesovarium are in continuity with the mesentery supporting the right lobe of the liver. In contrast, the mesorchium (or mesovarium) supporting the left adrenal continues ventrad and caudad as the mesorectum, and cephalad as the mesogaster.

Stroma

The adrenal gland is completely enclosed in a thin connective tissue capsule, which is in intimate contact with the supporting mesentery over most of its surface. Collagenous, reticular and elastic fibers are present in this capsule.

Collagenous fibers, when stained by Mallory's triple method, may be seen in the capsule as well as within the adrenal (fig.

5). Heavy bundles of collagenous fibers originating from the capsule surround the groups of radially arranged cells of the gland. Fine fibrils originating from these bundles pass among the cells. Extending from the wall of the large ventral vein a heavy network of fibers penetrates the gland. Collagenous fibrils are found in the walls of the vascular sinuses and small blood vessels. Many fibroblasts are found in the walls of these sinuses and vessels.

A striking picture is presented when the Foot-Menard method is used to demonstrate reticular connective tissue. Heavy black-stained bundles of reticular fibers extend inward from the capsule and are distributed throughout the gland. These bundles sharply demarcate the groups of cells that occur both in the chromaffin and the interrenal areas (figs. 4 and 10). Fine branching reticular fibers extend from these bundles and form a reticulum among the cells. Reticular connective tissue provides a much more extensive framework both around and within the groups of radially arranged cells than does collagenous connective tissue.

Short elastic fibers, stained by the Verhoeff-MacCallum method, are found both in the capsule and in the mesenteric support of the gland. Blood vessels show the typical arrangement of elastic fibers in the tunica adventitia. No elastic fibers are found either in the walls of the vascular sinuses or among the cells of the gland.

Melanophores are always present in the mesentery of the adrenal in the region adjacent to the dorsal body wall (fig. 2). In a few animals melanophores were found scattered throughout the entire supporting mesentery.

Parenchyma

General features. There is little variation in the gross morphology of mature alligator lizard adrenals. The adrenal is a composite organ consisting of an incomplete capsule of chromaffin cells intimately surrounding an elongated mass of interrenal cells (fig. 1). Heaviest concentrations of chromaffin

cells occur on the entire dorsal surface and around the caudal and cephalic poles of the gland. The chromaffin cells may extend deep into the interrenal portion and vice versa. Chromaffin cells may be intermingled either singly or in groups within the interrenal tissue near the center of the gland.

The low polygonal chromaffin cells are arranged radially around venous channels which permeate the gland. In many sections these cells appear to be irregularly arranged because of the collapse of vascular channels during fixation. However, when serial sections are examined, this radial arrangement is evident. In contrast the interrenal tissue in section shows a more orderly pattern of cell arrangement. The tall polygonal interrenal cells are arranged radially around the numerous vascular sinuses. Both collagenous and reticular connective tissue fibers demarcate this arrangement of the chromaffin and interrenal cells.

Chromaffin cells. When lizard adrenals are fixed in Zenker's solution containing the oxidizing substance, potassium dichromate, the granules in the chromaffin cells are colored brownish-yellow. In those cells having a greater concentration of granules, the coloration is more intense. Subsequent staining with Mallory's triple, Van Gieson's hematoxylin-azure II-eosin or iron hematoxylin accentuates this action of the fixative.

The Vulpian (1856) reaction clearly outlines the chromaffin tissue but with little variation in the intensity of coloration from one cell to the next (fig. 6). Chromaffin cells are colored a bright yellow when fixed by the Wiesel method. When stained with methylene blue-eosin the outer chromaffin cells appear brilliant green while the innermost zone takes on a deep blue.

The nuclei of chromaffin cells are relatively large and in section vary from a round to an oval shape and are usually located in the center of the cell. Nucleoli vary from one to three per cell. These stain bright red with anilin-acid fuchsin and deep blue with iron hematoxylin. Occasionally other granules not identified were seen in the nucleus.

Anilin-acid fuchsin stained the rod-like mitochondria as well as the spherical secretion granules. In most cells the mitochondria appear to be scattered evenly throughout the cytoplasm.

Golgi bodies stained by the da Fano method are frequently found concentrated near the nuclei of the chromaffin cells. In other cells the Golgi apparatus appears to be in a dispersed form. No polarity could be established for the Golgi apparatus.

Interrenal cells. The interrenal cells show some variations in staining intensity when fixed with Zenker-formol. The outermost interrenal cells stain much less intensely than do the innermost interrenal cells (fig. 1). Occasionally groups of lighter stained interrenal cells are found intermingled with more deeply stained cells. This variability in staining reaction was found with all cytoplasmic stains used.

Adrenals fixed with Zenker-formol-osmic acid show distinct rounded black droplets distributed uniformly within the interrenal cells (fig. 3). In Zenker-formol fixed preparations the periphery of the individual droplets often stains deeply with such cytoplasmic stains as orange G, methyl green and iron hematoxylin. The center of the droplet stains faintly giving the impression of a reticulum. These droplets may represent the lipoid interrenal secretion.

The shape and location of interrenal cell nuclei are varied. Some nuclei are spherical, located in the center of the cell and extend nearly across its breadth. Other nuclei are elongated due to compression. In cells adjacent to vascular sinuses, many nuclei are aligned close to the lumen of the sinus.

Nucleoli vary from one to 7 per cell. These are best demonstrated with either iron hematoxylin or anilin-acid fuchsin. Mitochondria in the interrenal cells are in the form of short rods and granules, the latter sometimes appearing to be joined by fine filaments. In all cells studied the mitochondria appear to be either concentrated close to the nucleus or widely scattered among the secretion droplets.

Golgi bodies may be identified in tissues prepared by the da Fano method. These structures appear either as a heavy network near the nucleus or in a dispersed granular form. No polarity for the Golgi apparatus could be established in these preparations.

Blood supply

Main arterial supply and venous drainage. The blood supply to the adrenal comes by way of numerous small arteries arising from the many large arteries in the vicinity, including the aorta, renal and mesenteric arteries.

The venous drainage of each adrenal passes by way of a large vein lying in contact with the ventral portion of the caudal half of the gland. On the right side this vessel drains into the postcaval vein. On the left side a similar vein is present which after receiving the venous blood from the gland crosses the midline of the body to join the postcaval on the right side, which in turn enters the right lobe of the liver.

Finer distribution of arteries and veins. Small arteries originating from the large arterial trunks penetrate the capsule and course immediately under the connective tissue but outside the parenchymal cells of the gland (fig. 1). These small arteries branch freely to form a subcapsular arterial plexus from which arterioles originate to supply the gland. Many small arteries of the subcapsular plexus first dip into the peripheral parenchymal cells and then return to the subcapsular level, with very few branches penetrating deeply into the gland (fig. 6). From the subcapsular arterioles numerous capillaries penetrate the chromaffin and interrenal tissues. In some areas these arterioles appear to open directly into vascular sinuses.

Within both the interrenal and chromaffin tissues of the gland there are numerous vascular sinuses variable in size (figs. 1 and 5). Venous drainage of the entire organ occurs by way of these vascular sinuses, some passing to the periphery of the organ and others coalescing to form one or more of the large central sinuses. These central sinuses drain directly

into the large ventral vein of each gland (fig. 5). Most of the sinuses which drain to the periphery (fig. 2) follow a course just under the capsule before making their way to the ventral vein.

No smooth muscle cells are seen in the walls of the vascular sinuses of the gland. These sinuses are clearly outlined by collagenous and reticular connective tissues.

Innervation

At the caudal end of the adrenal gland a large aggregation of sympathetic ganglion cells is found among the chromaffin cells. Similar nerve cells also occur either singly or in small groups scattered throughout the chromaffin tissue (fig. 9). The ganglionic cells are frequently found concentrated in the region of small arteries. These cells are clearly stained by methylene blue. From the ganglionic cells bundles of nerve fibers pass to the capsule of the gland; other nerves follow the course of the blood vessels and sinuses to be distributed throughout the entire adrenal.

Both chromaffin and interrenal tissues are innervated by these fibers passing from the capsule and blood vessels. Passing inward from the capsule, heavily stained bundles of nerve fibers course among the groups of both chromaffin and interrenal cells (figs. 7-8). The few nerve endings seen, appear as fine filaments terminating on the surface of both chromaffin and interrenal cells. Other fine fibers extending from the capsule pass among the cells to the blood sinuses.

DISCUSSION AND SUMMARY

There is little information on the histology of reptilian adrenals. These glands are usually described as consisting of chromaffin and interrenal cells irregularly intermingled. Although the turtle adrenal would answer to this description (Kuntz, '12) that of the alligator lizard, *Gerrhonotus multicarinatus*, would not. In this reptile the adrenal gland is a compound organ consisting of chromaffin cells intimately sur-

rounding an elongated mass of interrenal cells, with only slight intermingling of the two cell types. This arrangement is the reverse of that found in the adrenals of mammals where chromaffin cells form the medulla and interrenal cells the cortex.

The oxidative effect of chromium salts on chromaffin cells, known as the chromaffin reaction, is readily obtained in the lizard adrenal. Variations in this characteristic effect are accentuated by use of multiple cytoplasmic stains, and it may well be that these variations are correlated with stages in cell secretory activity.

Some variations in the colorations of interrenal cells are also shown by use of multiple stains. However, fixatives containing osmic acid most clearly demonstrate these variations from cell to cell by staining the lipoid secretion droplets black.

The low polygonal chromaffin and the tall polygonal interrenal cells are both arranged radially around vascular channels within the gland.

The stroma of the lizard adrenal consists of collagenous, reticular and elastic fibers. Although the capsule is composed of these three types of connective tissue, only collagenous and reticular fibers penetrate the glandular tissue proper.

The adrenal is an extremely vascular organ. Its arterial supply consists of an extensive subcapsular arterial plexus from which the blood passes into vascular sinuses either directly or indirectly by way of capillaries. Venous drainage is accomplished by way of a series of coalescing sinuses draining into a large ventral vein.

The intrinsic innervation originates from sympathetic ganglion cells lodged among the peripheral chromaffin cells. Nerve fibers from these cells pass to both the chromaffin and interrenal cells.

ACKNOWLEDGMENT

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PLATE 1

EXPLANATION OF FIGURES

1 Oblique section of Zenker-formol fixed adrenal differentially stained with Van Giesen's to show chromaffin cells (top) forming an incomplete capsule around the oval mass of interrenal cells. Note the large ventral vein (V.V.) and the subcapsular arterial plexus. A peripheral vascular sinus (V.S.) is present at bottom of figure. $\times 80$.

2 Transverse section showing association of adrenal with testis (T.). The testis has been rotated 45° downward during fixation. Figure is oriented with dorsal surface below. Note the continuous mesentery (M.) surrounding both the adrenal and testis. Pigmentation is evident in mesentery at right. Bouin fixative, Harris' hematoxylin-eosin. $\times 80$.

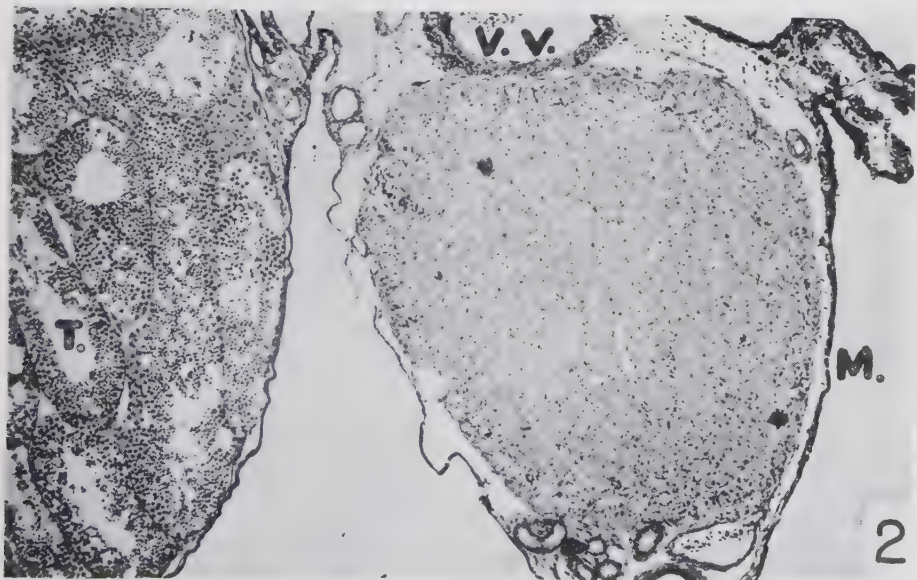
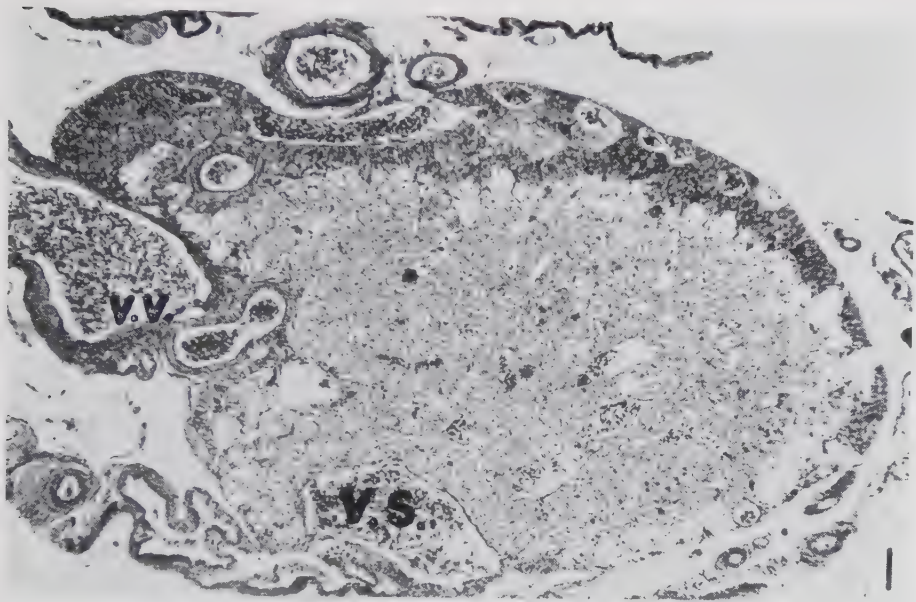


PLATE 2

EXPLANATION OF FIGURES

3 Transverse section of adrenal showing osmiophilic secretion droplets within the interrenal cells. Note less intense staining of chromaffin cells at right. Zenker-formol-osmic acid. $\times 60$.

4 Transverse section, near cephalic pole of adrenal, showing reticular connective tissue. Peripheral chromaffin tissue is deeply stained, and the reticular connective fibers (R.F.) are visible among both chromaffin and interrenal cells. Foot-Menard stain. $\times 60$.

5 Oblique section stained for collagenous fibers (C.F.). Note the large vascular sinus (V.S.) draining into the ventral vein at bottom of figure. Mallory's triple stain. $\times 60$.

6 Transverse section showing the Vulpian reaction in the chromaffin cells at right of figure. Note the arteriole penetrating the interrenal cells at bottom of figure. $\times 60$.

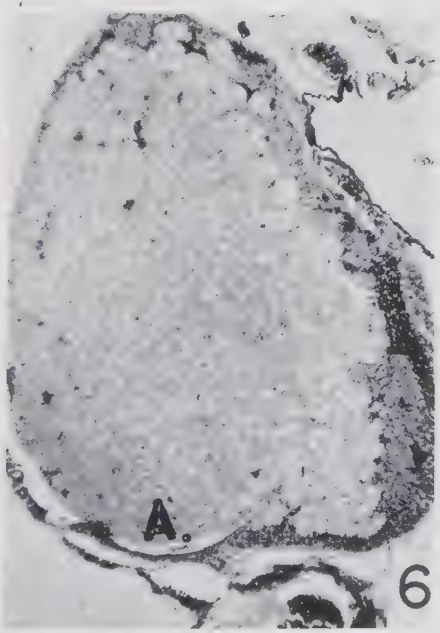
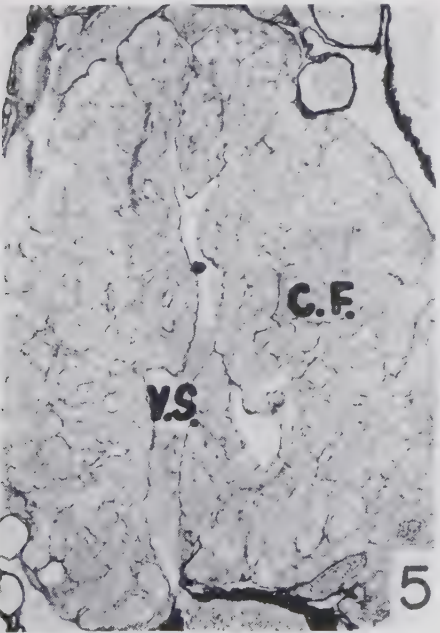
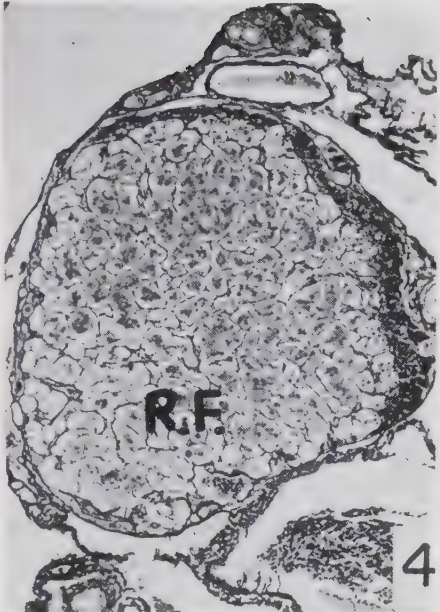
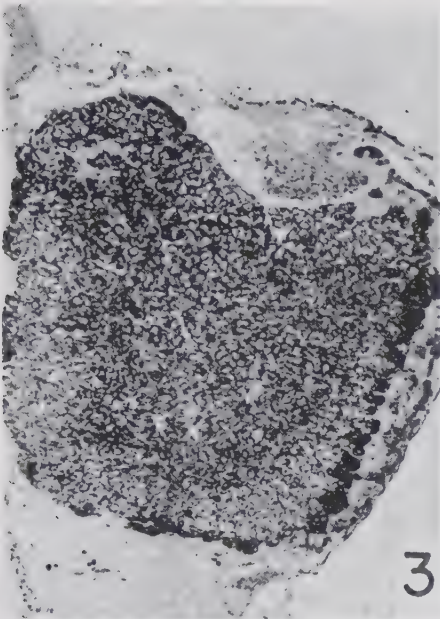


PLATE 3

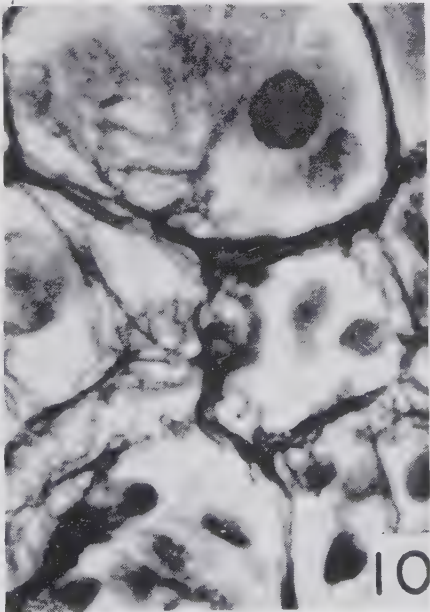
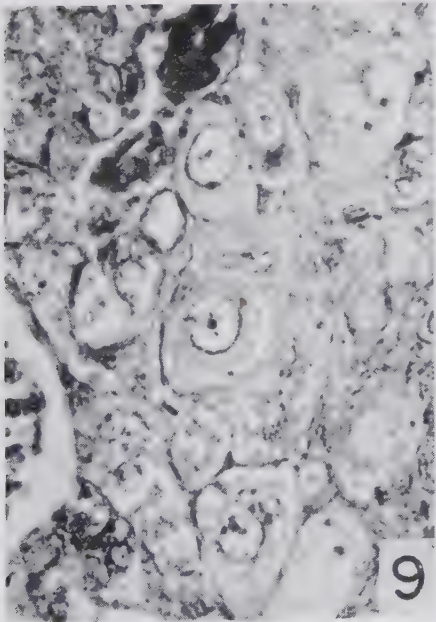
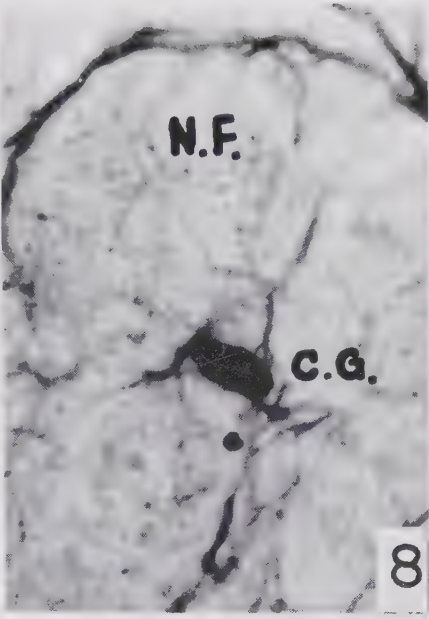
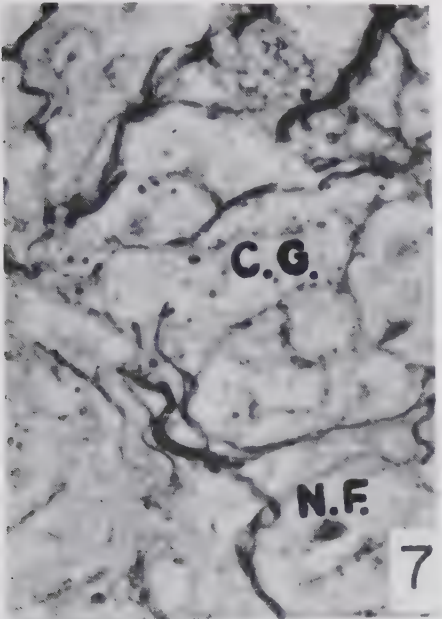
EXPLANATION OF FIGURES

7 Nerve fibers (N.F.) among the chromaffin cells. Near the center of figure note the small groups of cells (C.G.) outlined by nerve fibers. Pearson-O'Neill silver gelatine. $\times 1800$.

8 From same section as figure 7 showing a group of interrenal cells (C.G.) outlined by nerve fibers (N.F.). Pearson-O'Neill silver gelatine. $\times 1800$.

9 An aggregation of nerve cells in the chromaffin tissue at the caudal pole of the adrenal. Methylene blue-eosin. $\times 800$.

10 Reticular connective tissue fibers surrounding groups of interrenal cells. Foot-Menard stain. $\times 1800$.



THE ARCHITECTURAL PATTERN OF THE BOUNDARY BETWEEN EPITHELIUM AND CONNECTIVE TISSUE OF THE GINGIVA IN THE RHESUS MONKEY ¹

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FOURTEEN FIGURES

INTRODUCTION

The morphology of the boundary between epithelium and connective tissue of the gingiva has been extensively studied in histologic sections. However, such two-dimensional pictures of the complex patterns of these tissues are sometimes difficult to interpret, and it was felt that a three-dimensional study of this region would clarify the histologic picture.

The object of this investigation was to study the architectural pattern of the boundary between epithelium and connective tissue of the gingiva in the rhesus monkey. In order to arrive at a true three-dimensional picture of this complicated surface, a method was employed which to the best of our knowledge had not been used before for the oral mucosa; namely, the separation of connective tissue and epithelium. The contact surface could then be studied on both tissues and the findings correlated with the appearance of the boundary in histologic sections.

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³ Part of a dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science in Histology at the University of Illinois.

REVIEW OF LITERATURE

It has been known for many years that in the skin the epidermis can be readily separated from the dermis by treatment with dilute acetic acid. Baumberger, et al., ('42) believed Menschel ('25) to be the first to accomplish this, but the method is actually much older. As long ago as 1883, Unna described a technique for separation of the epidermis from the dermis using dilute citric or formic acids, and this technique was probably first used considerably earlier. Cowdry ('38) fully described a method for stripping off the epidermis by the use of acetic acid, and a technique for its subsequent staining and mounting. Boiling water has also been used to obtain separation (Harris, '27).

Baumberger, et al., ('42) discovered that by placing a section of skin on a slide warming table at exactly 50°C. for a few minutes, the epidermis could be peeled off with forceps. If, in this experiment, the skin was allowed to cool again before attempting separation, a firm union between the two layers was found to have been re-established, which seemed to indicate that a reversible gel-sol transformation was taking place at the boundary between the epithelium and connective tissue. Invariably the area of epidermis was greater than that of the dermis from which it had been removed.

Felsher ('47) showed that separation can also be produced by certain neutral salts at neutral pH. The ease of separation seemed to be correlated with the amount of swelling produced in commercial leaf gelatin by the salt used.

MATERIAL

The present study is based upon the investigation of 60 gingival biopsy specimens from 12 living rhesus monkeys (*Macaca mulatta*) and on histologic studies of the jaws of two rhesus monkeys which had died following acute infections. The ages of these animals ranged from approximately 18 months to 5 years. The biopsies were taken from various gingival regions of both jaws anterior to the first permanent

molars, the animals being under intraperitoneal nembutal anesthesia (1.0 cm³ of a 3% nembutal solution per kilogram of body weight).

The animals were maintained on a diet of fresh lettuce, apples, bananas and carrots, augmented by bread and milk. Their gingival condition was good, although some showed slight inflammation of the interdental papillae in the upper incisor region.

METHODS

Various known methods for the separation of the epithelium from the lamina propria were tested on the gingival biopsy specimens, using a dissecting microscope and fine tweezers. The maceration technique by means of 0.25% or 0.5% acetic acid produced the best results in 6 hours at room temperature.

Eight of the separated specimens were fixed in 5% formalin, sectioned by the paraffin method and stained with hematoxylin and eosin (fig. 3). This allowed histologic study of the level of separation.

In order to check whether the entire epithelial attachment of the gingiva to the tooth was being obtained in the biopsy specimens, the following experiment was made. The deciduous incisors and gingivae were removed en bloc from the upper and lower jaws of a young living monkey approximately 18 months old. The epithelium was then removed from both the lamina propria and the teeth by the acetic acid method. A comparison of the epithelial attachments obtained in this manner with those from the biopsy specimens indicated that, in many instances, the entire epithelial attachment was being obtained by the biopsy method.

Photographs of the specimens immersed in glycerine were taken immediately after separation. Oblique illumination from a single source was used.

For the serial sections, the jaws were fixed in 5% formalin and decalcified in 5% nitric acid. Selected blocks contained the upper incisors from one animal, and the lower incisors

and canines from the other. After embedding in celloidin, each of these blocks was divided and then sectioned serially, one half in a transverse and the other in a frontal plane. Every 5th section was stained with hematoxylin and eosin. Other sections were stained with silver and Mallory's stain.

TOPOGRAPHY OF THE GINGIVA

For the purpose of orientation the gingiva of the rhesus monkey can be divided into three areas, following the classification of the human gingiva suggested by Orban ('48) (fig. 1).

(a) *The free gingiva.* This portion surrounds the tooth like a collar and is not directly attached to the tooth surface.

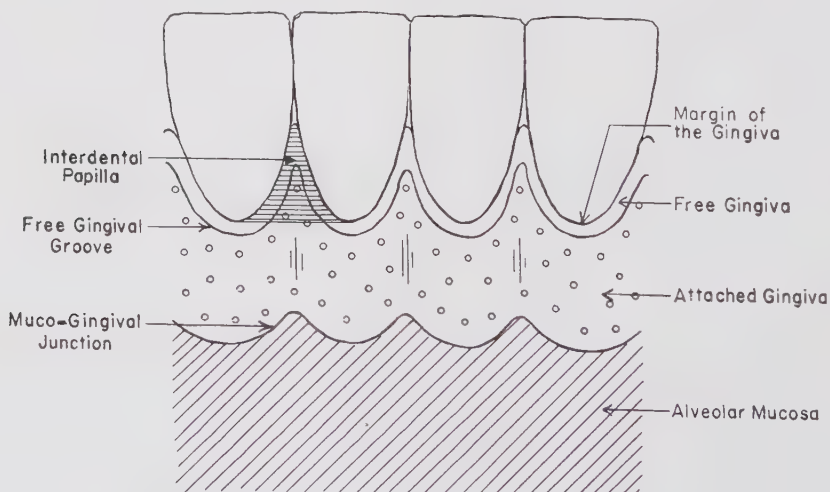


Fig. 1 Diagram showing the different regions of the human gingiva (Orban, '48).

It forms the outer wall of the gingival sulcus and also extends from the bottom of the gingival sulcus to the coronal border of the gingiva (margin of the gingiva).

(b) *The attached gingiva.* This portion extends from the border of the free gingiva to the muco-gingival junction, where it joins the alveolar mucosa. On the palatal side of the maxilla, the gingiva joins the palatal mucosa. On the lingual side

of the mandible the gingiva joins a narrow area of alveolar mucosa, which is continuous with the sublingual mucosa. A shallow groove, the free gingival groove, is usually found at the junction of the free and attached gingiva.

(c) *The epithelial attachment.* This portion of the epithelium is organically attached to the surface of the tooth. Depending upon the amount of epithelial downgrowth which has occurred, this attachment may be to enamel only, enamel and cementum, or cementum only.

The interdental papilla is that part of the gingiva which is situated between two adjacent teeth. Because of the presence of a diastema the interdental areas between the upper latter incisor and canine, and between the lower canine and the first premolar, do not form interdental papillae.

The alveolar mucosa is that portion of the oral mucosa immediately adjacent to the gingiva. In the palate the palatal mucosa is adjacent to the gingiva.

OBSERVATIONS

A. *Alveolar mucosa*

The loosely attached alveolar mucosa presented the simplest arrangement of the boundary. The epithelium could easily be separated from the lamina propria. The ease of separation was probably due to the fact that in this region the connective tissue papillae were comparatively few in number, short and slender. The connective tissue had a fairly flat surface, and contained low, regularly spaced parallel ridges. Most of these ridges ran in a vertical direction, and were surmounted by the conical papillae. The papillae numbered approximately 110 per square millimeter.

Viewed from the under side, the epithelium presented the appearance of a flat sheet, pitted in a regular pattern (fig. 4). Shallow grooves in the epithelium, corresponding to the low ridges in the connective tissue, connected the pits. No epithelial pegs were present.

After separation the area of the connective tissue appeared to be slightly smaller than that of the epithelium in this region.

Serial sections through the upper jaw (fig. 5) showed the thin epithelium and relatively few connective tissue papillae in the alveolar mucosa.

B. Attached gingiva

At the border between the alveolar mucosa and the attached gingiva, the boundary between the epithelium and connective tissue presented an abrupt change. The papillae of the connective tissue were greater in number and longer and wider than those of the alveolar mucosa (figs. 6 and 7). The bases of the papillae were usually connected by low ridges, running more or less vertically, which caused the papillae to appear as if arranged in rows. Some of the papillae were divided near their ends into two or more small secondary papillae. The average number of papillae in the attached gingiva was 170 per square millimeter.

The epithelium showed a corresponding increase in the number, diameter and depth of the pits, which produced an appearance similar to a honeycomb (fig. 6). The pits or "cells" of this honeycomb were mostly square or rhomboidal in shape, and were separated by ridges running generally in a vertical and horizontal position. The vertical ridges were usually slightly higher than the horizontal ridges. Some of the "cells" of the honeycomb were subdivided into smaller pits close to their blind ends. Occasionally an epithelial ridge was divided by one or two transverse notches. The short parts of the ridge so formed resembled epithelial pegs.

The surface area of the lamina propria and the epithelium at the boundary appeared to be similar in size after separation, in contrast to the finding in the alveolar mucosa.

On the palatal side of the upper jaw, the epithelium of the attached gingiva was thicker than in other regions and contained deeper and wider epithelial ridges (fig. 11).

Tangential and horizontal sections (figs. 8 and 9) through the attached gingiva demonstrated the epithelial honeycomb and the epithelial ridges. In the tip of the connective tissue papillae only two capillaries, i.e., one capillary loop, were present (fig. 8). The wider bases of the papillae contained more capillaries.

C. Free gingiva and epithelial attachment

1. *Outer surface.* As the gingival margin was approached, the connective tissue papillae instead of projecting at right angles from the surface of the lamina propria, were inclined toward the margin. At the actual margin the main connective tissue ridges became more pronounced and changed direction, running parallel to the margin. The connective tissue papillae surmounting these ridges were much smaller at the gingival margin and occasionally were absent for short distances. The epithelium showed the normal honeycomb pattern, which changed to form parallel ridges at the gingival margin (fig. 6). The sulci between these ridges contained shallow pits for the connective tissue papillae.

No change of the boundary between epithelium and connective tissue could be discerned at the region of the junction of the free and attached gingiva (free gingival groove) in cases showing no clinical signs of inflammation. In the regions of the diastema, the boundary between epithelium and connective tissues was similar to that of the attached gingiva.

2. *Dental surface (gingival sulcus and epithelial attachment).* In cases which showed no clinical signs of inflammation the apical border area of the epithelial attachment presented itself as a smooth, but narrow strip. However, at some slight distance from the apical border of the epithelial attachment pits for connective tissue papillae could be found (fig. 2), arranged, on an average, in 4 or 5 rows. The pits, which were deep and close together in this region, were not at right angles to the surface of the tooth, but ran almost parallel to its long axis. Thus, their general direction was

toward the gingival margin. In some specimens the connective tissue papillae were so close together that they left very little room for the epithelium between them. Here, the epithelium appeared to hang from the marginal area like curtains of irregular length. Near the gingival margin these curtains were so split by the connective tissue projections that they

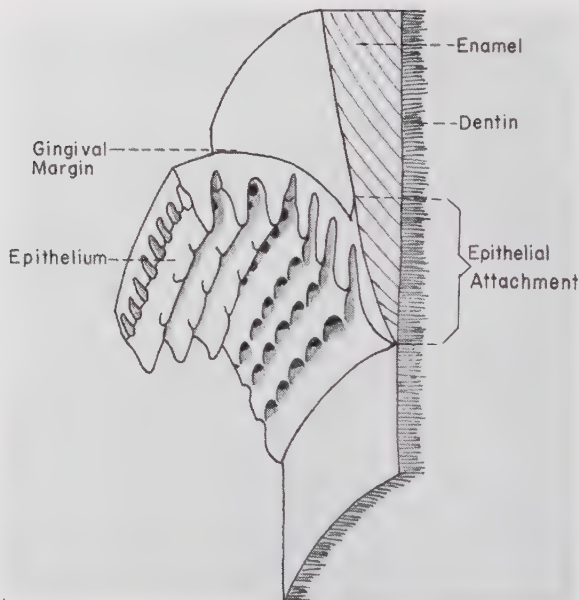


Fig. 2 A diagrammatic representation of the free gingiva and epithelial attachment after the connective tissue had been removed. Note the pits in the epithelial attachment running almost parallel to the long axis of the tooth, and the pits in the sulci between the epithelial ridges at the gingival margin.

did actually form epithelial pegs in many cases. This is the only region in the gingiva where epithelial pegs, as such, were often found.

A horizontal section near the tip of an interdental papilla (fig. 10) showed on one side cross sections through connective tissue papillae in the epithelial attachment, and on the opposite side similar papillae in the epithelium of the free gingiva facing the tooth.

3. *Interdental papilla.* In the region of the crest of the interdental papillae the main epithelial ridges sometimes formed a whorl (fig. 6), the connective tissue ridges between having short, secondary conical papillae. The boundary between epithelium and connective tissue of the gingival sulcus and epithelial attachment in this region was similar to that described above.

D. Palatal mucosa

The transition from the gingival zone to that of the mucosa of the hard palate was not as marked as that between the gingiva and the alveolar mucosa. The ridges of the palatal connective tissue were closely set together, and ran mostly at right angles to the palatal rugae. Short conical papillae projected from the crests of these ridges, and numbered approximately 200 per square millimeter. The epithelium differed from the gingival epithelium by having shorter and narrower ridges, set more closely together, with the transverse ridges scarcely developed (fig. 11). The total thickness of the epithelium was less than that of the attached gingiva.

In a section through the palatal mucosa (fig. 12) the large number of epithelial ridges in this region was apparent. The epithelium was, however, not as thick as it appeared in this section cutting through the epithelium in an oblique direction.

E. Areas of chronic gingival inflammation and of repair

All the animals examined showed some degree of inflammation of the interdental papillae between the upper central incisors. The interdental papillae appeared slightly edematous and red, with a glazed surface.

The greatest change of the boundary between epithelium and connective tissue in the inflamed tissue was found at the tip of the interdental papilla (fig. 13). Here, some of the epithelial ridges had proliferated and grown down more deeply into the connective tissue. The downgrowth of certain of the

epithelial ridges produced an irregular and coarser epithelium honeycomb, with a few large "cells" containing many subdivisions close to their blind ends. The connective tissue showed the usual multitude of conical papillae on its surface, and troughs for the enlarged epithelial ridges.

Farther away from the tip of the interdental papilla, where less inflammation was noted clinically, the honeycomb pattern of the epithelium was preserved. The ridges of this honeycomb were thicker and blunter than those in areas free of any inflammation, however. The vertical ridges were much more prominent in these regions, and in a few places short epithelial pegs arose from their edges. Although the pattern of the connective tissue papillae appeared to be normal, the individual connective tissue papillae were narrower than those in uninfamed areas.

In the regions of inflammation, the epithelium facing the tooth (sulcus epithelium and epithelial attachment) was found to have a greater area than normal. The pattern of the boundary between epithelium and connective tissue, however, was similar to that of the normal areas.

Regions from which biopsies had been taken approximately one month previously were found to be completely healed. Biopsy of the healed areas revealed that the boundary between epithelium and connective tissue showed the normal fine honeycomb pattern in the epithelium.

DISCUSSION

A. The possible mechanism of separation. The ease of separation of the epithelium from the connective tissue following maceration in various solutions is paralleled by the degree of swelling of collagen or gelatin produced by these solutions (Baumberger, et al., '42; Felsner, '47). However, this observation does not prove that the swelling is responsible for the separation of these tissues. In histologic specimens stained with silver, Mallory's or the Hotchkiss method ('46), collagenous or reticular fibers in the basement membrane are rarely seen actually to touch the epithelial cells of the basal

layer. Between the fibers and the basal cells there is normally an optically homogeneous component of the basement membrane which consists of ground or cementing substance. This ground substance is closely related to that found in all connective tissue (Gersh and Catchpole, '49).

Thus, the separation may be the result of the conversion of the ground substance forming this homogeneous layer of the basement membrane from a water-insoluble to a water-soluble state. This change could be caused by a depolymerization of the ground substance.

B. The architecture and function of the boundary between epithelium and connective tissues. The simplest pattern of the boundary would seem to be that of the alveolar mucosa. Here, the connective tissue had a fairly flat surface, containing extremely low and regularly spaced parallel ridges surmounted by short conical papillae. The epithelium appeared as a flat sheet with shallow grooves connecting the pits.

At the transition from alveolar mucosa to attached gingiva an increase in all the dimensions and in the number of the connective tissue papillae altered the appearance of the boundary between epithelium and connective tissue, forming in the epithelium a "honeycomb" pattern. The "cells" of this honeycomb were square or rhomboidal in shape. This modification permitted a greater area of vascular connective tissue to be in contact with the basement membrane beneath the epithelial cells, and enabled capillaries to run close to this membrane right to the tip of the papillae. This would insure better nutrition demanded by increased mitotic activity in this epithelium for the replacement of desquamated cells lost by the friction of mastication, and the biochemical changes of cornification. The firmness of the attachment of the epithelium to the connective tissue would also be enhanced by this modification of pattern.

As the free gingiva was approached, the shallow vertical ridges connecting the bases of the connective tissue papillae often became accentuated, and continued, usually without any alteration, into the free gingiva. At the gingival margin they

changed direction and ran parallel to this margin. A corresponding accentuation of the vertical ridges of the epithelial honeycomb, with a similar change in direction at the gingival margin, was observed. The vertical ridging probably served a mechanical function by providing additional resistance to shearing forces tending to separate the epithelium from the lamina propria during mastication. The palate was also well provided with such ridges. The greater depth of the horizontal ridges at the gingival margin may be caused by "wrinkling" under the forces of mastication.

C. Comparison with the skin. A comprehensive study of the variations in the architectural pattern of the boundary between epithelium and connective tissue in different regions of the skin could not be found in the literature. It would appear, however, that modifications in the pattern similar to those in the mucous membrane of the mouth are found in the skin (Hoepke, '27). Thus in regions not normally subjected to severe mechanical stress the corium forms a sheet with regularly arranged conical papillae. Shallow ridges connecting the bases of these papillae may or may not be present. In regions of the skin exposed to great stress, ridges at the boundary between epithelium and connective tissue are well developed. Thus, in the palms of the hands and soles of the feet, alternate parallel ridges of the epithelial honeycomb are deepened. Corresponding to these deepened internal ridges, external ridges cause the complicated pattern of "dermatoglyphics." The alternate, shallower epithelial ridges of the boundary between epithelium and connective tissue correspond to the sulci between the external ridges.

Previous writers have remarked that when the epidermis and dermis of skin are separated there is a difference in the area of the boundary between epithelium and connective tissue of the two tissues. The fact that this phenomenon was not observed in the gingiva could be accounted for by the absence of elastic fibers in the lamina propria of the gingiva. Thus, after separation, the lamina propria does not tend to contract as it does in the dermis. In the region of the

alveolar mucosa where elastic fibers are numerous, the connective tissue was found to contract slightly on separation.

E. Epithelial attachment. In every case where the teeth had erupted into functional occlusion, the epithelial attachment was found to be pitted for the reception of connective tissue papillae. From this we might deduce that the epithelial attachment, when more than a few cell layers thick, required connective tissue papillae for its nourishment.

In man, the normal epithelial attachment has been claimed to possess a smooth boundary between epithelium and connective tissue, free of peg formation. Thus, Gottlieb ('44) stated: "Ordinarily the epithelial attachment has no pegs, such as are common to epithelium in other places, e.g., on the epithelium of the gingiva. When peg formation is present on the epithelial attachment, it is a sign of proliferation from irradiation."

Yet, in this study of the rhesus monkey, in every case, the epithelial attachment was pitted by many connective tissue papillae, running practically parallel to the long axis of the tooth. Only the most apical portion of the epithelial attachment was normally free of connective tissue papillae, and the epithelium appeared as a narrow smooth sheet, independent of the degree of inflammation. This smooth area was not more than a quarter of the total area of epithelium facing the tooth, that is, the epithelium of the gingival sulcus and epithelial attachment. In the separated specimens it was not possible to measure the depth of the gingival sulcus, but clinical examination before biopsy, and a study of decalcified sections has shown the gingival sulcus to be of minimal depth in these animals.

It would, of course, be premature to relate the findings obtained from the rhesus monkeys directly to the human gingiva. However, from the study of a few separated specimens from human biopsy material, it would seem that the architectural pattern of the boundary between epithelium and connective tissue is similar to that of the rhesus monkey.

E. Epithelial pegs. One of the more striking findings of this investigation was the rare occurrence of epithelial pegs or papillae in the gingiva. A peg, by definition, is a small, usually cylindrical, pointed or tapered object. This term would be applicable to the papillae of the connective tissue which fit into the pits of the epithelium. Corresponding epithelial projections, however, were rare, though sections of epithelial ridges may give the appearance of pegs.

It is suggested that in the description of gingival sections, the term "epithelial peg" or "epithelial papilla" should be abandoned unless it has been determined by serial sections or by the separation method that the epithelial projection in question, is, in fact, of conical shape, and is not a cross-section of a ridge. In general, the epithelial extensions into the papillary layer of the lamina propria should be described as "epithelial ridges."

F. Appearance of epithelial ridges in sections. It became also evident from a study of the separated gingival epithelium that caution must be exercised before attaching any importance to appearances in sections such as "spiking", "blunting", "rounding" or "clubbing" of the epithelial ridges. All these variations in the epithelial pattern could be produced by making sections through a normal epithelial honeycomb at different angles to the line of the ridges (fig. 14).

In this study it was noted that a widening of the epithelial ridges and an alteration in the design of the honeycomb may be found in regions of inflammation. But a few sections of such an area in which the plane of section might pass through the main ridge at any angle, or be mainly parallel to these ridges, would be difficult to interpret. In fact, the study of a few such sections might lead to a totally erroneous concept of the architecture of the boundary between epithelium and connective tissue in the region under examination.

In a few specimens the epithelial ridges of the free and attached gingiva became confluent and formed an epithelial dome projecting into the connective tissue (fig. 6). These com-

paratively rare findings usually were found in the center of the epithelial "whorls" in the interdental papillae.

Epithelial "domes" would not seem to be important signs of gingival inflammation. They were found in regions which showed no clinical signs of inflammation. A section passing through such a "dome" produces a rather unusual appearance and has been accorded pathological significance (Zizkin, et al., '36).

G. Changes in chronic inflammation. King ('44) on the basis of sections of decalcified ferret's jaws with varying degrees of gingival disease, stated: "The foregoing illustrations have demonstrated the displacement of the fibrous tissue and other components of the corium by proliferating epithelial *papillae* (italics ours). He believed these changes to be similar in many ways to those of certain types of gingival disease in man. In the same paper he described a "bushing" of the peripheral capillaries in the lamina propria, as seen with a capillary microscope in living and dead animals. The "bushing" was, in his view, a fairly early sign of gingival pathology.

In the present study it was found that in regions showing clinical signs of chronic inflammation in the gingiva of the rhesus monkey, some of the ridges had proliferated and extended deeply into the lamina propria. The normal fine epithelial honeycomb was changed into an irregular pattern with much larger honey-comb cells containing the smaller normal cells as subsidiary pits.

SUMMARY AND CONCLUSIONS

The present study was based on the investigation of 60 gingival biopsy specimens taken from 12 living rhesus monkeys, and of parts of the jaws of two other animals removed at autopsy.

The architectural pattern of the boundary between epithelium and connective tissue in the gingiva was examined with a dissecting microscope, after the two tissues had been separated by maceration in 0.5% acetic acid for 6 hours.

Findings on serial sections of the decalcified jaws from the autopsy specimens were correlated with direct observations made on the separated tissues.

The findings were as follows:

1. The loosely attached alveolar mucosa had the simplest pattern of the boundary between epithelium and connective tissue. The epithelium was a flat sheet, pitted at intervals by short, conical connective tissue papillae.

2. In the attached gingiva the connective tissue papillae were larger in size and more numerous per unit area. This produced a "honeycomb" appearance in the epithelium, with more prominent vertical ridges.

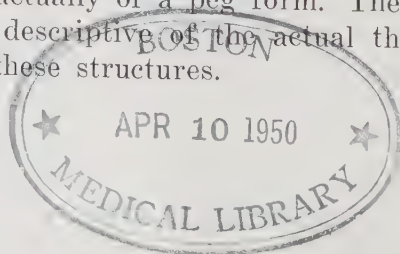
3. In the free gingiva, the epithelial honeycomb was similar to that of the attached gingiva. At the gingival margin the horizontal ridges of the honeycomb were more prominent than the vertical ridges and ran parallel to the margin.

4. The epithelial attachment of teeth in functional occlusion was pitted by connective tissue papillae. The direction of these papillae was almost parallel to the long axis of the tooth.

5. The palatal mucosa showed well-developed ridges in the epithelium and connective tissue, although these ridges were smaller and more closely arranged than in the attached gingiva. Pits in the troughs between the epithelial ridges were always present. They were produced by papillae arising from the crest of the connective tissue ridges.

6. In mild chronic inflammation, the main ridges of the epithelium were thickened, but with a greater degree of inflammation a downgrowth of some of the epithelial ridges into the connective tissue had occurred.

7. Epithelial pegs or epithelial papillae were rarely found in the gingiva. Therefore, use of such terms as epithelial pegs or papillae should be abandoned unless it has been determined that any particular epithelial process seen in a section is actually of a peg form. The term epithelial ridges is more descriptive of the actual three dimensional appearance of these structures.



8. Such appearances as "blunting", "spiking," "rounding," or "clubbing" of epithelial processes in sections might be produced by various planes of sectioning of the epithelial honeycomb. Caution should be exercised before attaching any particular importance to such findings when only one or two sections are available.

ACKNOWLEDGMENTS

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PLATE 1

EXPLANATION OF FIGURES

3 Photomicrograph of a gingival biopsy from a young adult monkey. The epithelium (A) was separated from the lamina propria (B) after treatment with 0.5% acetic acid for 6 hours. Note that separation had occurred at the basement membrane. Stained with hematoxylin and eosin. $\times 180$.

4 Photograph of the under surface of separated epithelium from a biopsy of the alveolar mucosa of the lower central incisor region of a young adult monkey. The area of the biopsy is indicated in the insert. The epithelium appears as a flat sheet containing regularly arranged pits (a) which are connected by shallow grooves (b). $\times 40$.

5 Photomicrograph of the alveolar mucosa in a horizontal section through the upper jaw of a young adult monkey. Note the thin epithelium and relatively few connective tissue papillae. Stained with hematoxylin and eosin. $\times 130$.

6 Photograph of the under surface of the separated epithelium (A) and the surface of connective tissue (B) of an interdental papilla obtained in a gingival biopsy from a young adult monkey. The area of the biopsy between the lower central incisors is indicated in the insert. Note in (A) the epithelial honeycomb of the attached gingiva (a), the parallel ridges of the marginal area (b), the pits in the epithelial attachment (c), the epithelial whorl (d), and the epithelial dome (e). $\times 40$.

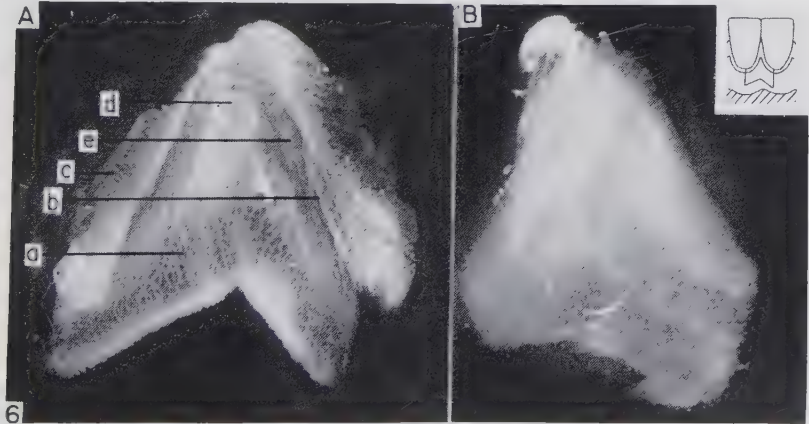
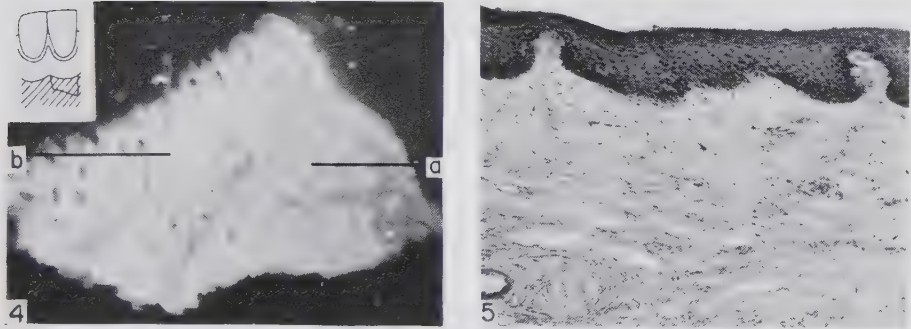
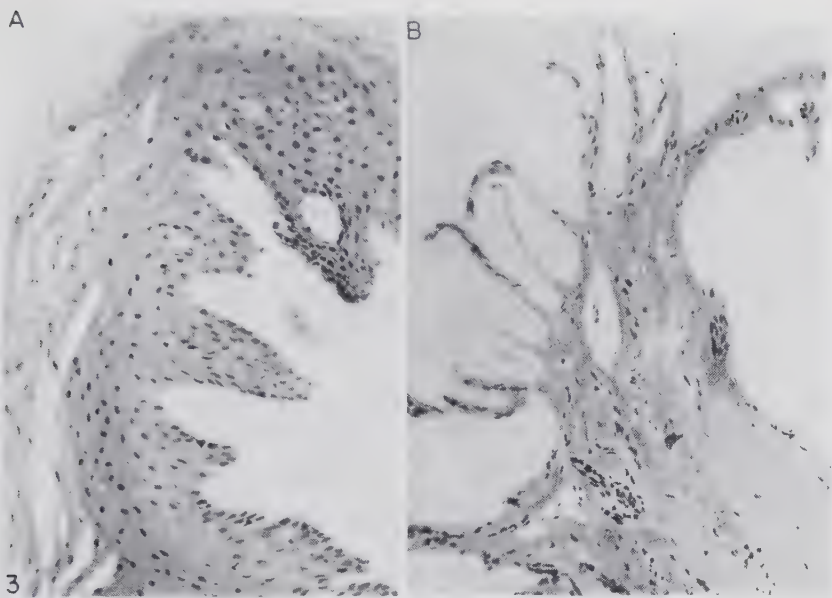


PLATE 2

EXPLANATION OF FIGURES

7 Photograph of the lamina propria of an interdental papilla from a biopsy of the palatal side of the upper incisor region of a young adult monkey. The area of biopsy is indicated in the insert. Note the large number of fine conical connective tissue papillae. $\times 30$.

8 Photomicrograph of a tangential section through the attached gingiva of a young adult monkey. Note the islands of connective tissue (a) surrounded by a network of epithelium (b). Silver impregnation. $\times 130$.

9 Photomicrograph of a horizontal section through the upper jaw of a young adult monkey, showing the attached gingiva of the labial side of an interdental papilla. Note the thickness of the epithelium (a) and the number of epithelial ridges (b). Stained with hematoxylin and eosin. $\times 130$.

10 Photomicrograph of a horizontal section through the upper jaw of a young adult monkey, passing through an interdental papilla near the tip. Epithelium was attached to the enamel (a) (lost in preparation) on one side. There is calculus (b) in the pocket on the other side. Note the large number of connective tissue papillae (c) in the epithelium (d) on both sides. Silver impregnation. $\times 130$.

11 Photograph of the under surface of the epithelium from a biopsy of the palatal mucosa and attached gingiva of a young adult monkey. The area of the biopsy is indicated in the insert. Note the well-developed ridges (a) which become finer in the palatal mucosa (b). $\times 20$.

12 Photomicrograph of an oblique section through the palatal mucosa of a young adult monkey. Note the large number of epithelial ridges (a). Stained with hematoxylin and eosin. $\times 130$.

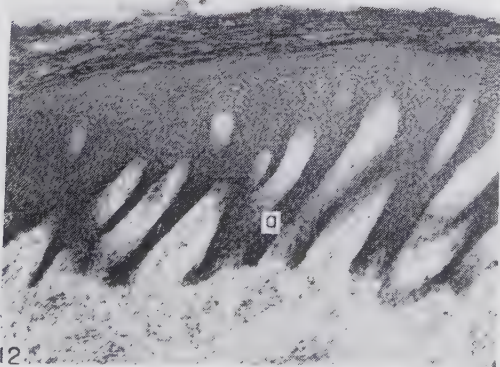
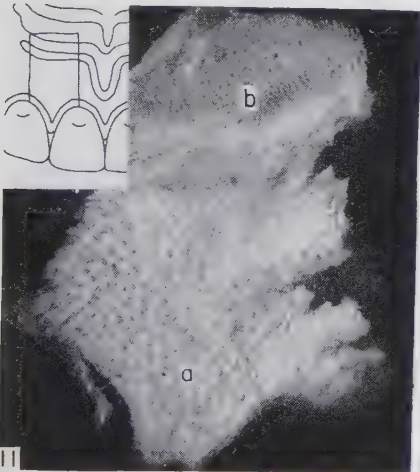
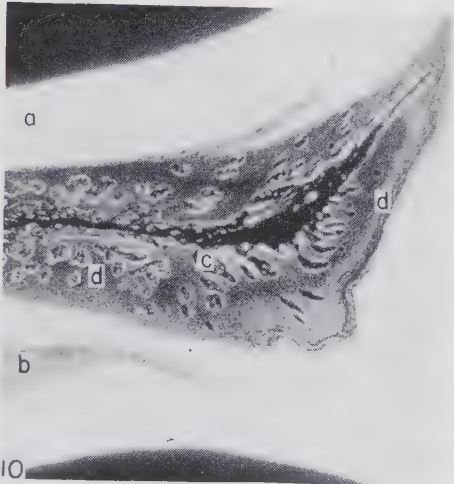
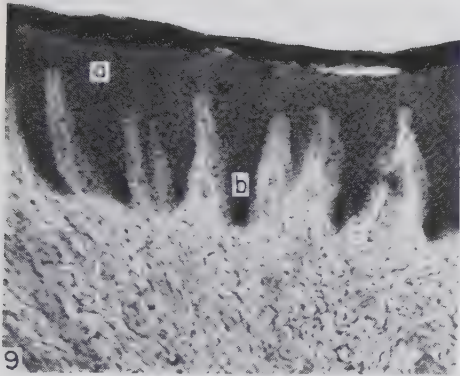
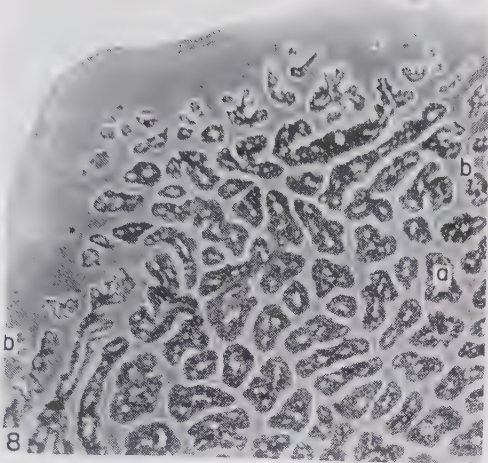
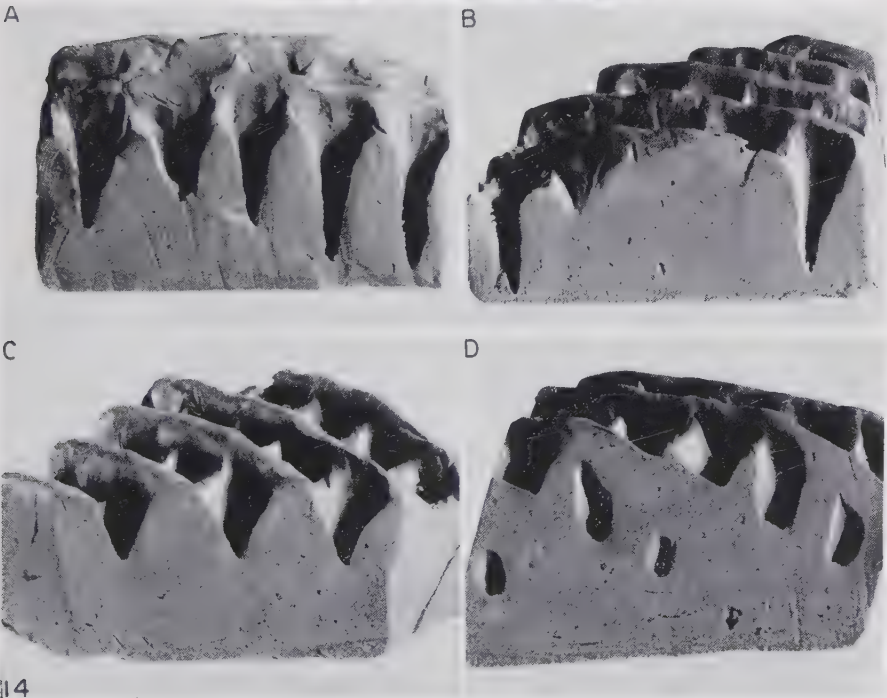
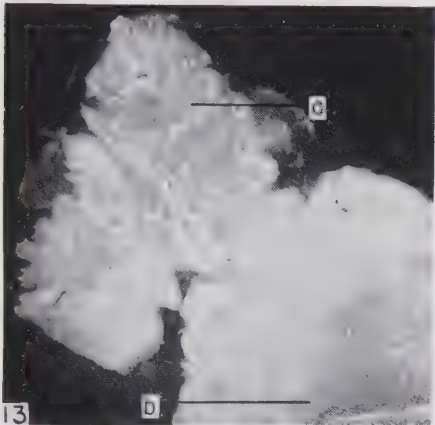


PLATE 3

EXPLANATION OF FIGURES

13 Photograph of the undersurface of the epithelium from a gingival biopsy of a chronically inflamed interdental papilla from between the upper central incisors of a young adult monkey. The region of the tip of the papilla (a) shows a coarse honeycomb pattern, the large "cells" being subdivided into smaller pits at their blind ends. Further away from the source of the irritation, a honeycomb, which is normal except for thickened ridges (b) is seen. $\times 20$.

14 A plasticine model of an epithelial honeycomb similar to that found in the attached gingiva. This model has been sectioned in various planes and shows some of the different pictures which may be obtained. In (A) the line of section runs at right angles to the surface, parallel to the main epithelial ridges, and practically midway between two of them. B shows a similar section at right angles to the surface, but cutting obliquely through one of the main epithelial ridges. C shows a section at right angles to the surface, but cutting through the main epithelial ridges at 45° . D shows a section at 45° to the surface, and also cutting obliquely through the main epithelial ridges.



ELECTRON MICROSCOPE STUDIES ON THE STRUCTURE OF CARDIAC MUSCLE^{1, 2}

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SIXTEEN FIGURES

INTRODUCTION

The application of the electron microscope to cytological and histological studies depends largely on being able to reduce the material sufficiently thin to permit penetration of the electrons. For most cells and tissues this may be said to be in the order of 0.1 to 0.3 μ . Fortunately, striated muscle fibers may be satisfactorily reduced to smaller units, the myofibrils and myosin filaments, which are admirably suited for electron microscope study. This is evidenced by the beautiful electron micrographs taken of them by Hall, Jakus and Schnitt ('46). Shadow casting and layer stripping have also been successfully used in connection with electron microscope studies on muscle (Reed and Rundall, '48). Muscle has likewise been used in connection with investigations of thin sectioning techniques, suitable for electron microscope studies (Richards, Anderson and Hance, '42; Sjöstrand, '43). Pease and Baker ('48) have developed these methods, and have applied them in connection with a revealing study on the structure of striated muscle (Pease and Baker, '49). Kisch, Grey and Kelsch ('48), studied heart tissue by means of a similar technique. They were especially interested in the Purkinje fibers, of which a further discussion will be given below.

¹ We wish to express our appreciation to Prof. H. E. Jordan of the University of Virginia for certain suggestions and for kindly reading the manuscript.

² This investigation has been aided by a grant from the Iowa Division of the American Cancer Society, and by a grant from the U. S. Public Health Service (administered by J. H. Bodine).

Our interest in making this study was twofold: (1) to determine whether information could be obtained on cardiac muscle structure supplementing that already known, and (2) to compare the structure of cardiac muscle with the structure of skeletal muscle as revealed by the light microscope (Jordan, '20a, b; '33; and others) and as revealed by the electron microscope (Hall, Jakus and Schmitt, '46; Schmitt, Bear, Hall and Jakus, '47; Pease and Baker, '49). As pointed out by Hall, Jakus and Schmitt ('46) little new information of skeletal muscle structure has actually been revealed by the electron microscope except for the demonstration of the continuity of the myosin filaments. However, it may be said that this technique confirms and yields clearness of detail of muscle striations superior to that of any other method.

No effort will be made to refer to all of the extensive literature on cardiac and skeletal muscle. For a detailed description of the structure and reference to the literature on cardiac muscle see Jordan ('11, '17, '20a, b, '33), Jordan and Banks ('17) and Cohn ('32). For similar papers dealing with skeletal muscle see Jordan ('20 b, '33) and Barer ('48).

MATERIAL AND METHODS

The material used in this study consists of the ventricular myocardium and part of the auricle of the guinea pig heart. It was slightly stretched and fixed in 10% formalin while taut. The muscle was then cut into small pieces and washed in water and placed in a Waring blender where it was allowed to agitate for 20 minutes. The time was divided into 5 minute intervals to prevent excess heating of the material. It was then allowed to stand until the heavier masses of the muscle had settled to the bottom of the tube, leaving the smaller groups and isolated fibers suspended. Light centrifuging of the material was also used to hasten the action of gravity in displacing the larger masses of the muscle. The suspension containing the myofibrils was then placed on the electron microscope grid that had been previously coated with celloidin. The fibers were then stained with 0.1%

phosphotungstic acid, according to the method of Hall, Jakus and Schmitt ('46). The electron microscope used was a R.C.A., Type EMU-2B, equipped with unbiased gun. The approximate magnification of the micrographs may be determined from the $1\ \mu$ scale on each figure.

RESULTS AND DISCUSSION

I. The structure of cardiac muscle as revealed by the light microscope

The cytology of cardiac muscle as seen under the light microscope is too well known to warrant a detailed description of it here. Both Jordan ('33), and Cohn ('32) have discussed at length the structure, terminology, and literature relative to this subject. It will suffice to mention here that cardiac muscle fibers, like those of skeletal muscle, are composed of smaller units known as myofibrils. These structures are divided transversely into anatomical and physiological units known as sarcomeres. The sarcomeres are repeated longitudinally throughout the myofibril in a metameric fashion. Each sarcomere in turn may show a stratification of substances, the appearance of which varies with the state of contraction or relaxation of the fiber. The striations have been referred to as bands, discs and membranes and have been identified by the letters J(I), Z, N, Q(A), H, E, and M. These striae are all illustrated in figures 1, 2 and 3 and will be referred to in detail below.

It is known that mammalian cardiac muscle closely resembles skeletal muscle in so far as the number and positions of the striations are concerned. It is usually stated, however, that certain of the bands are not clearly defined in cardiac as they are in skeletal muscle.* Cardiac muscle displays an additional structure, the intercalated disc, which has been the subject of much discussion among histologists. This deeply staining band occurs at intervals along the fibers and is thought to be one of the three distinguishing features of the mammalian myocardium; the position of the nuclei, branching

and syncytial nature of the fibers being the others. The intercalated disc has been variously interpreted: as an artifact, as representing cell boundaries, as growth centers and as irreversible contraction bands (Jordan, '47).

II. *The structure of the myofibril*

The myosin filament. Jordan ('20a) in examining the wing muscle of the wasp, observed that the myofibrils were composed of smaller elements which he termed metafibrils (see also Heidenhain, '11). Striking electron micrographs of skeletal muscle myofibrils of frog and rabbit have been shown to contain continuous myosin filaments which course uninterruptedly through at least several adjacent sarcomeres (Hall, Jakus and Schmitt, '46). The myosin filaments remain relatively straight regardless of the state of contraction or relaxation of the muscle. They may sometimes show a beaded appearance in both the J(I) and Q(A) bands. The bundles of myosin vary from about 50 to 250 Å in width and possess indefinite length. It is not clear from the paper by Hall, Jakus and Schmitt ('46) whether or not the myosin filaments occur in single units or in small groups. In any case, they are considered to be the contractile units of the muscle.

In a recent publication, Pease and Baker ('49) concluded (as did Marcus, '25) that the myofibril of rat striated muscle is composed of an aqueous core surrounded by a denser wall. The evidence for this was obtained from electron micrographs of cross sections of muscle and from the appearance of air bubbles in the core when lipolized muscle was immersed in a viscous fluid. The core of the myofibril is thought to contain protein substance; the wall, the myosin filaments and the "A substance" of Hall, Jakus and Schmitt ('46). Pease and Baker ('49) further suggest that the myofibril contains a variable number of compartments which may account in part for the longitudinal striations seen in muscle. They interpret the electron micrographs of Hall, Jakus and Schmitt ('46) as representing only the wall, instead of the whole myofibril.

It will be observed that the electron micrographs in figures 1, 2 and 3 compare favorably with those of rabbit skeletal muscle taken by Hall, Jakus and Schmitt ('46). It is not possible from such preparations to determine with certainty the form of the myofibril; however, it appears to be rounded or ribbon shaped. The latter appearance may be due in part to drying. In any case, it is difficult to agree with Pease and Baker ('49) that such figures represent only the walls, rather than the whole of the myofibrils. The fine filaments (myosin filaments) seem to extend uninterruptedly throughout the length of the portions of the myofibrils shown in figures 1, 3 and 4. They are especially clear on either side of the Z membrane in the region of the N band. Here small groups of the myosin filaments seem to form bundles of a larger size, which are probably comparable to the metafibrils referred to by Jordan ('20a). The single myosin filaments described by Hall, Jakus and Schmitt ('46) are in the order of 50 to 250 Å in width, and the metafibrils shown in certain of our preparations are in the order of 200–300 Å in width. Whether or not the myosin filaments are distributed singly, or in small groups within the living myofibril cannot be answered from our study.

Figure 4 probably represents two myofibrils that have been laterally stretched and at the same time have lost their striated appearance except for the Z and M membranes. The groups of myosin filaments that have been partially separated laterally reveal short interconnections between them. It may be that this intermetafibrillar substance represents only partially separated myosin filaments or some form of interfibrillar "cement." In this same figure the bundles of myosin filaments can be traced as continuous longitudinal elements throughout at least two or more sarcomeres.

The questions concerning the distribution of the myosin filaments within the myofibrils is difficult to answer from our material. Certainly they appear to be located near the surface of the myofibril (fig. 1). Here, in the relatively clear intermetafibrillar spaces of the N band, no underlying layers of the metafibrils are evident. If this is true, one would ex-

pect to find some differentiation between the wall and the center of the myofibril. Such a condition might be interpreted as present in the form of a darkened core-like region in figures 5 and 6 and in the upper portion of the myofibril shown at left in figure 7. Certainly figure 5 seems to show convincing evidence for the presence of a core within the myofibril, but it may actually be an artifact (e.g., plane of focus, differential staining or some electron optical effect). Kisch, Grey and Kelsch ('48) have also observed a similar darkened "core" surrounded by a lighter area in certain connective tissue fibers. Because of the work of Hillier and Ramberg ('47), they are inclined to interpret such figures as a kind of Fresnel fringe effect due to astigmatism of the lens.

The Z membrane. The Z membrane which has a high affinity for certain stains bounds the sarcomere on both ends (fig. 1). Evidence from the histological studies (Jordan, '20b) and from electron micrographs (Hall, Jakus and Schmitt, '46; and Pease and Baker, '49) indicate that the Z membrane extends between the myofibrils and binds them together. Speidel ('39) has observed this membrane in living muscle but was unable to decide whether or not it functions in holding the myofibrils together. Barer ('48) presents evidence against the existence of a Z membrane or other transverse membranes uniting the myofibrils. He suggests that the alignment of the myofibrils is maintained by surface forces, long-range colloidal forces, or possibly hydrogen bonds.

The evidence from our electron micrographs of the Z membrane supports the view that it is a relatively elastic structure which extends from one myofibril across the interfibrillar sarcoplasmic region to the neighboring myofibril where it is continuous with the Z membrane of the same level. This is especially well illustrated in figure 9 where the myofibrils are only slightly separated. The Z membrane, while laterally stretched, still retains its continuity between the fibers. Where the myofibrils have been further separated, the Z membranes are broken, leaving laterally extended edges which are evidence of their former connections (fig. 7). If this membrane

possesses elasticity this would allow for considerable variation in alignment of the myofibrils, the latter being one of the main objections to the view that the myofibrils are attached at the Z membranes.

Hall, Jakus and Schmitt ('46) describe the Z membrane as being an amorphous substance which binds the myosin filaments together in this region. On the other hand, Pease and Baker ('49) contend that the Z membrane is extrafibrillar and, therefore, confined only to the sarcoplasmic spaces.

In figures 1, 7 and 9, the Z membranes appear sharply outlined and, therefore, must be relatively dense or have a considerable affinity for the stain. Here (figs. 1 and 9) the edges of the membrane are not perfectly smooth but appear somewhat serrated at points of contact with the myosin filaments. A similar but more exaggerated condition of the same effect is noted in the lower somewhat torn Z membrane of figure 11. Such figures may be interpreted as showing a close association of the Z substance with the myosin filaments. Further evidence of the intimate relationship of the Z membrane to the myosin filaments is seen in figure 4. The myosin elements are laterally stretched and partially separated in the region of the M membrane; however, in the region of the Z membrane the separation of them is not so obvious. Hence, we are inclined to agree with Hall, Jakus and Schmitt ('46), that the Z membrane aids in the alignment of the myosin filaments.

It has been suggested from time to time that the Z membrane is a double structure. In the contracted myofibril of figure 5, a partial fracture is evident in the region of the Z membrane near the mid-point of the figure. This being a contracted myofibril, a contraction band is present at this point in addition to the Z membrane. Accordingly, while the fracture may be exactly in the middle of the Z membrane, the dark areas on either side of the break probably represent in part the two halves of the contraction band. It has often been noted by histologists that the muscle fibers tend to fracture at the level of the Z membrane. With this we agree +

(fig. 4), but we have also noted frequent breaks in the myofibrils at the level of the N disc (fig. 6).

The M membrane. The general opinion seems to be that the M membrane which bisects the Q disc is a structure much more delicate, yet fundamentally like, the Z membrane. Accordingly, it has been assigned the function of binding the myofibrils together in much the same way as does the Z membrane. As with the Z membrane, Pease and Baker ('49) consider it to be extrafibrillar in position. They have made the interesting observation that at certain points along the fiber, the Z and M membranes seem to join, which might account for the vernier type shift in bands sometimes seen in muscle.

In our material the M membrane is distinctly shown in figures 1, 2, 3, 4 and 5. The metafibrils definitely pass longitudinally through it and appear to be held together by it. It cannot be categorically stated whether the M membrane is an intra- or extramyofibrillar structure or both; however, it seems to be in intimate contact with the myosin filaments.

The Q band. As pointed out by Hall, Jakus and Schmitt ('46), since the myosin filaments run uninterruptedly through the sarcomeres, the relatively dense Q(A) band must be due to some accessory substance which they have termed the "A substance." There is evidence that calcium, magnesium and potassium are chiefly concentrated in this region, especially in relaxed muscle (Schmidt, Bear, Hall and Jakus, '47). In addition, the Q band is relatively anisotropic as compared to the J band. Electron micrographs of layer-stripped muscle show the Q band to be raised above the level of the J band which is interpreted to indicate that a greater amount of solid matter is present in Q than in the J band (Reed and Rundall, '48). It has been established by histological methods and by the use of the electron microscope that at the time of contraction the deeply staining substance of the Q band divides in the region of the M membrane and moves in opposite directions, eventually reaching the opposing Z membranes. Here it meets a similar half of the migrating Q band from the adjacent sarcomere. This results in the for-

mation of a contraction band. This condition we have been able to confirm (compare figures 1, 2 and 5). We can add little to the description of this band in cardiac muscle beyond that given for skeletal muscle by Hall, Jakus and Schmitt ('46).

How the substance of the Q band is confined within the myofibril, and the nature of its movement upon contraction and relaxation of the myofibril, is difficult to understand since the myofibril appears to possess no well defined membrane. If limited to the periphery of the myofibril, it could conceivably move during muscle action through the space between the myosin filaments (fig. 1).

The H band. In the central region of the Q band, on either side of the M membrane, may be seen a lighter area which is known as the H band, or median disc of Hensen (figs. 1 and 2). This band cannot be clearly observed in a fully relaxed fiber (fig. 11). The appearance of the band signifies an initial contraction associated with the movement of the Q substance from the median region of the sarcomere in the direction of the opposing Z membranes.

The X band. Hall, Jakus and Schmitt ('46) report that the "myofibrils from rabbit muscle usually show two to four fine cross bands symmetrically disposed with respect to the M membrane and near it." These lines, they state, are beyond the resolution of the light microscope and they interpret them as periodic variations in the density of the A(Q) band. We have frequently observed on either side of the M membrane, and placed symmetrically to it, rather faint but well defined stripes which, to our knowledge, have not been described heretofore for cardiac muscle (X in figs. 2 and 3). We believe these lines to be consistent components of guinea pig cardiac myofibrils and propose to refer to them tentatively as a new band, designated by the letter X. This same stripe is apparently present in figure 2b of the paper of Hall, Jakus and Schmitt ('46).

It is not our purpose, in describing this stripe as a new one, to complicate further the literature of muscle cytology. However, if its appearance in electron micrographs proves

relatively consistent, as seems to be the case in our material, it will be desirable to have available a suitable term to identify it. Its relative position in relation to the M membrane is comparable to the relative position of the N stripe to the Z membrane. As Hall, Jakus and Schmitt ('46) suggest, it may only represent a periodic variation of the Q substance. However, much the same could be said for the presence or absence of the H band. It should be added that we have never observed the number of similar lines (two to 4) in this region as mentioned by Hall, Jakus and Schmitt ('46). However, further work may demonstrate their presence.

The J band. The J or light band which is bisected by the Z membrane and contains two N bands is said to be relatively isotropic. As mentioned before, the myosin filaments pass uninterruptedly through it. Hence, it would seem that both the J and Q bands are composed of fundamentally the same contractile protein. Dempsey, Wislocki and Singer ('46), find that the J and Q bands stain differently with acid and basic dyes at different hydrogen ion concentrations. In addition, they state that the J band is argyrophilic following the Bodian protargol method. Caspersson and Thorell ('42) have shown that the strongly ultraviolet light absorbing substances (adenylic acid and adenylypyrophosphoric acid) are chiefly located in the isotropic segment of the resting insect muscle. They think of the J band as the seat of the chemical energetic processes, the Q band as the seat of the contractile elements.

The J band is clearly outlined in figures 1, 2 and 3. In figure 1, especially, the metafibrils or groups of myosin filaments are clearly displayed running parallel to each other in the direction of the long axis of the myofibrils. It is not known whether the grouping of the myosin filaments into bundles, with clear spaces between them, represents the condition in life. If it represents the true structure (not artifact), the clear areas might conceivably represent the paths for the movement of the Q substance at the time of contraction of the muscle.

The N band. The appearance of the N band depends to a large extent on the degree of contraction or relaxation of the myofibrils. It is present at N in figures 1, 2 and 3. In the contracted fibril of figures 5 and 8, it has either been displaced or masked by the movement of the Q substance.

Pease and Baker ('49) report that the N band is extra-fibrillar and that it exists in the form of a collar around the myofibril. The substance of the N band would appear (figs. 1, 2 and 3) to be closely associated with groups of myosin filaments. The spaces between the myosin filaments in this region (fig. 1) have been observed by Pease and Baker ('49) in contracted fibers. They interpret them as due to the N substance having moved out, or having become dispersed, as a result of contraction.

As mentioned above, the myofibril often fractures in the region of the N band. An interesting differential in size of two parts of the same myofibril is seen in the region of the N band of figure 6. This may be due to a differential swelling or shrinking of the two parts of the fibril before or at the time of fixation.

The E band. Electron micrographs of guinea pig cardiac muscle also show the E band between the Z membrane and the N band (figs. 1 and 2). Cohn ('32) states that "the N and E discs are relatively speaking unimportant and, on the whole, inconstant in human heart muscle." We cannot agree with the latter statement, at least for guinea pig cardiac muscle, because both bands appear consistently in suitable preparations.

It is apparent that the myofibrils shown in plate 1 display all the bands, stripes and membranes previously described for skeletal and cardiac muscle.

III. *The intercalated disc*

The intercalated disc is usually thought to be characteristic of cardiac muscle. However, Jordan ('19) has presented good evidence to show that it may also be found at times in skeletal muscle. According to Jordan, it has been variously

interpreted as an intercellular cement substance, as regions of muscle growth, as tendons, and as irreversible contraction bands. It may appear as a straight, step-like or serrated band, and is said to be chiefly peripheral in position, extending for varying depths, but never completely through a fiber.

The dark band at I.D., in figure 11, we have interpreted as a rather simple form of an intercalated disc for the following reasons: (1) it is present at the region of the Z membrane, (2) it is larger than the neighboring Z membranes, and (3) since the myofibrils are in a relaxed state it cannot represent a simple contraction band. In other words, its appearance here seems to support the interpretation of Jordan ('33) that intercalated discs represent irreversible contraction bands. They were not often found in our preparations, possibly because the myofibrils fracture easily at this region.

IV. Miscellaneous observations

Under this heading we propose to show certain structures that were observed from time to time while exploring the grid for typical cardiac myofibrils. Lack of pure preparations of these various structures make final identification of them difficult. However, we are including them here in the event that they may serve as a stimulus for further work in this field.

"*Muscle clot.*" Speidel ('38, '39) has carefully described and discussed the literature on muscle clotting. He has demonstrated the formation of muscle clots upon injury and various other types of stimulation. From the description of muscle clots by Speidel and others it would appear that the myofibril in figure 10 probably shows such a condition. If true, the myofibril was injured in some way at or just before the time of fixation. Figures 14 and 16 represent two myofibrils found in the same field. They are rather dark and show considerable variation in the height of the dark and light bands. Figure 16 is interesting because apparently the material of the dark bands has been removed on the part of the myofibril at the left, leaving a goodly portion of it free of the "outer" material. The question which immediately arises here is,

does the material that has been removed represent the Q and J substances or does it represent only a thinning out of these substances due to a stretching or retraction within the fibril? Could it be that the part which has been removed represents the "wall," and the part remaining, the "core" of the myofibril?

"Purkinje fiber components." Purkinje fibers are usually described as differing from cardiac muscle fibers in being thicker and showing fewer striations. Kisch, Grey and Kelsch ('48) saw in human cardiac muscle, by aid of the electron microscope, large dense fibers which they interpreted as Purkinje fibers. These contain, in addition to muscle, straight dark fibers which they tentatively interpreted as conductive tissue. Segmentation of the conductive tissue was not observed, but it was surrounded by a network of thin connective tissue fibers.

Figure 13 shows structures which we are tentatively interpreting as part of the conductive tissue of the heart. These column-like units measure about 250 Å in width and 3 μ in length. They are unsegmented, possess relatively smooth borders, and apparently are associated with a dense form of connective tissue. It is suggested that these may actually represent the units of structure of the conductive fibers of the heart in much the same way as the myofibril is the unit of structure of the striated muscle fiber.

"Connective tissue." Kisch, Grey and Kelsch ('48) state that they were unable to demonstrate in ventricle-cardiac tissue the segmented type of connective tissue fibers. However, this was not true for the connective tissue associated with the Purkinje fibers.

In what appears to be connective tissue elements of the heart, we, too, have been unable to see the typical banded condition that occurs in collagen fibers (Hall, Jakus and Schmitt, '46). The fibril in figure 15 is in order of 100 Å in width and displays consecutive enlargements along its longitudinal axis. If not collagenous, it must represent either an elastic or reticular fiber.

"Nerve components." From the works of De Robertis and Schmitt ('48a, b) it seems plausible that the structures illustrated in figure 12 represent some component of the nerve fiber. We have not observed the cross banding of these units as reported for some neurotubules by De Robertis and Schmitt. However, they state that "in single electron micrographs, frequently all transitions may be noted between neurotubules which show dark edges but a clear core devoid of cross-bands and those which, in addition, show cross-bands with great clarity."

In addition to branching, the structures shown here fit the description given for neurotubules by De Robertis and Schmitt ('48a, b). They do not appear to represent connective tissue elements.

SUMMARY

All the striations previously described for cardiac muscle have been demonstrated in single myofibrils. These include the J, Z, N, Q, H, M and E. In addition, a stripe appearing on either side of the M membrane has been observed. This apparently has not been previously described for cardiac muscle. It has been designated by the letter X. It may represent only a variation in the substance of the Q disc as mentioned by Hall, Jakus and Schmitt ('46). However, its presence seems relatively consistent in cardiac myofibrils, which alone seems sufficient reason to justify a tentative term to designate it for future reference.

The myofibrils vary in width from about 2 to 3 μ . They appear rounded or possibly ribbon shaped. Groups of myosin filaments were observed extending uninterruptedly in relatively straight lines throughout the length of at least two adjacent sarcomeres. The myosin filaments appear to be grouped in bundles (metafibrils) which are located near the surface of the myofibril. No clear evidence was found for the presence or absence of a central aqueous "core." Darkened core-like structures were observed in some myofibrils, but these may only represent artifacts due to some unknown electron optical effect. However, in any case we are inclined

to agree with the view of Pease and Baker ('49) to the extent that some degree of differentiation exists between the central and the more peripheral regions of the myofibril. No evidence for a limiting membrane around the myofibril was observed.

Z membranes are relatively dense structures which extend continuously across adjacent myofibrils and function in binding them together at this region. They probably also aid in aligning the groups of myosin filaments in this region. The M membrane is present in cardiac muscle. Its structure and function, although less marked, is probably similar to that of the Z membrane.

The Q band probably represents some material distinct from the myosin filaments. It has a relatively high density and shifts from its position near the middle of the sarcomere in relaxed myofibrils to the position of the opposing Z membranes upon contraction of the myofibrils. The path of movement taken by the Q substance during muscle action could conceivably be within the spaces between the groups of myosin filaments. The H band appears upon contraction of the myofibril and movement of the Q substance toward the opposing Z membranes.

In the relaxed myofibril the J band is bisected by the Z membrane and it also includes the two N bands of adjacent sarcomeres. In contracted myofibrils, the N band is often masked due to the accumulation of the Q substance from adjacent sarcomeres (contraction bands) at this region. Fixed myofibrils fracture more often in the regions of the Z membrane and N band than they do elsewhere.

Evidence was obtained to support the view of Jordan ('33) that the intercalated disc probably represents an irreversible contraction band.

Other structures that have been tentatively interpreted as muscle "clots," units of the conductive fibers of Purkinje, "neurotubules" and connective tissue elements have been illustrated.

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PLATE 1

EXPLANATION OF FIGURES

Scale: 1μ

1 Myofibril showing one sarcomere. All membranes and bands previously described for cardiac muscle are evident. Groups of myosin filaments (metafibrils) are clearly illustrated in the region of the N band. Inter-metafibrillar spaces also are shown in this region.

2 and 3 In addition to the striations shown in figure 1, an additional stripe (X) may be seen on either side of the M membrane.

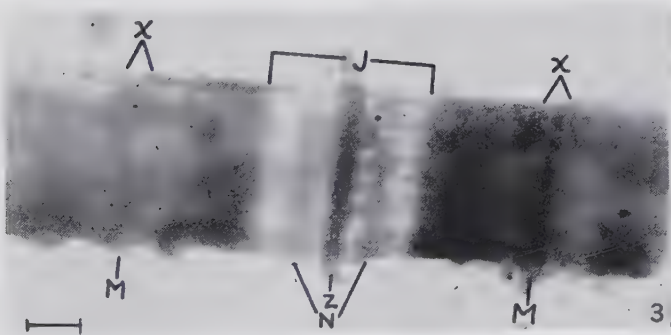
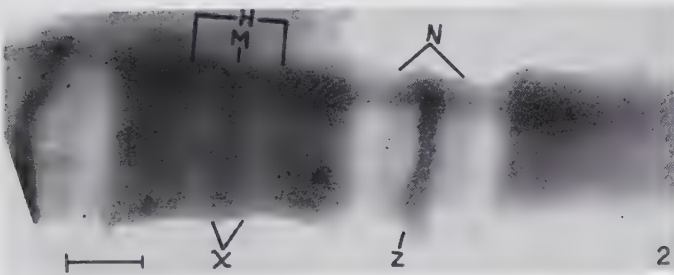
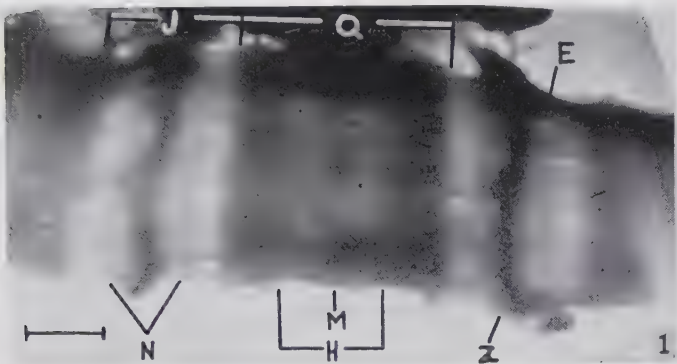


PLATE 2

EXPLANATION OF FIGURES

4 Apparently, two adjacent myofibrils laterally stretched. Separation of the groups of myosin filaments in the region of the Q band with fine connections shown between them is seen. Note also the continuity of the groups of myosin filaments extending throughout the sarcomeres.

5 Contracted myofibril with contraction bands (C, B) and a partial fracture in the region of the centrally located Z membrane. A centrally located darkened "core" is apparent.

6 Myofibril showing a fracture in the region of the N band. A difference in size of the two parts of the myofibril is evident. As in figure 5, a darkened central region is present.

7 Separated myofibrils showing lateral extension of the Z membrane. Upper portion of left myofibril displays a darkened region in the center.

8 Contracted myofibril with contraction bands.

9 Two myofibrils partially separated. Here the laterally stretched Z membrane extends across the inter-myofibrillar space.

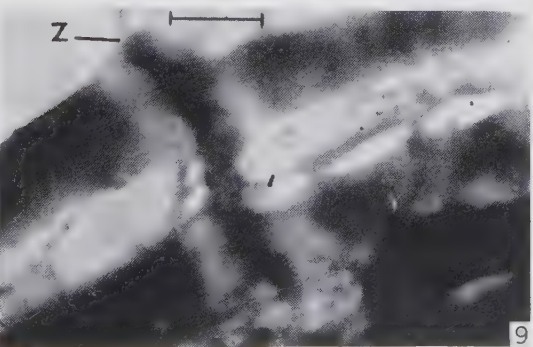
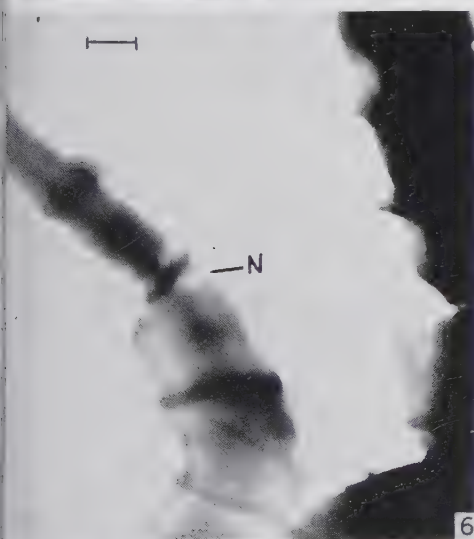
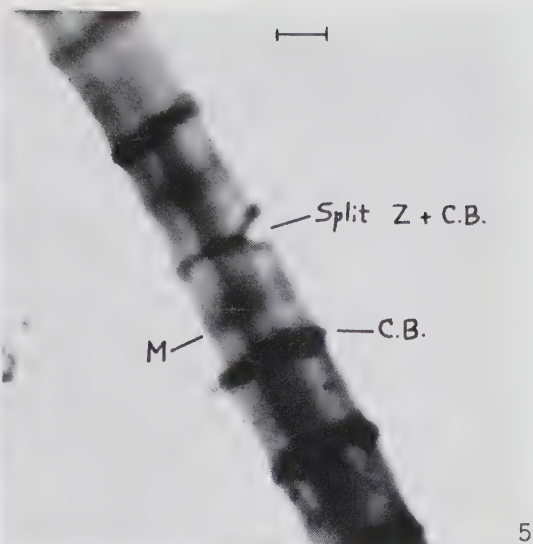
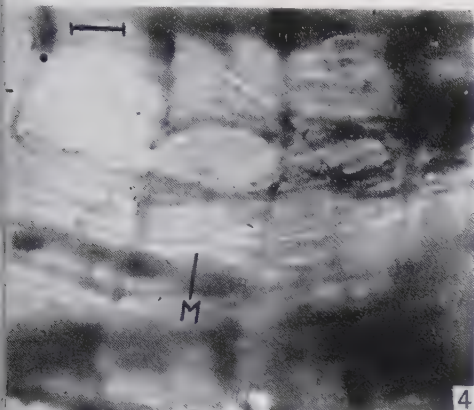


PLATE 3

EXPLANATION OF FIGURES

10 Probably a muscle "clot."

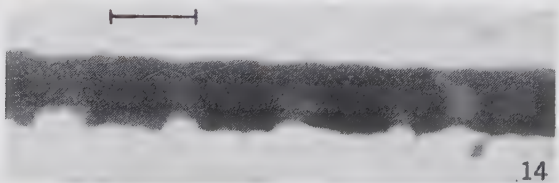
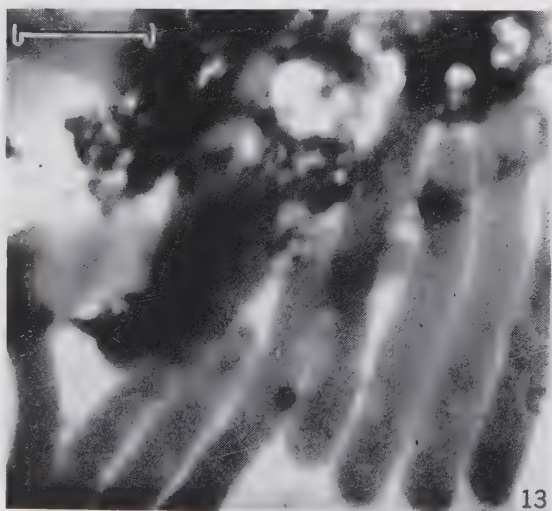
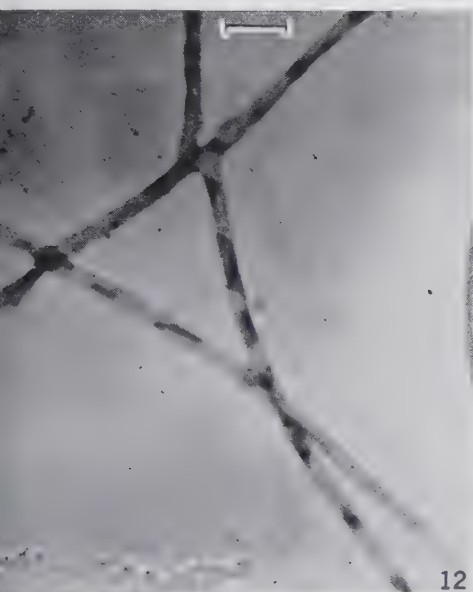
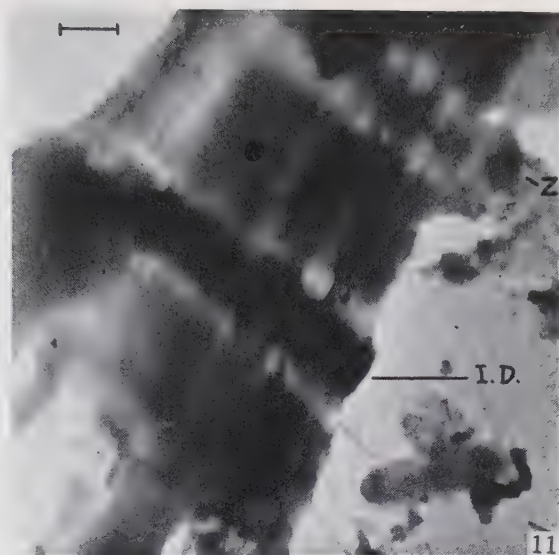
11 Group of myofibrils showing intercalated disc (I.D.). A longitudinally stretched and torn Z membrane is evident near the bottom of the figure.

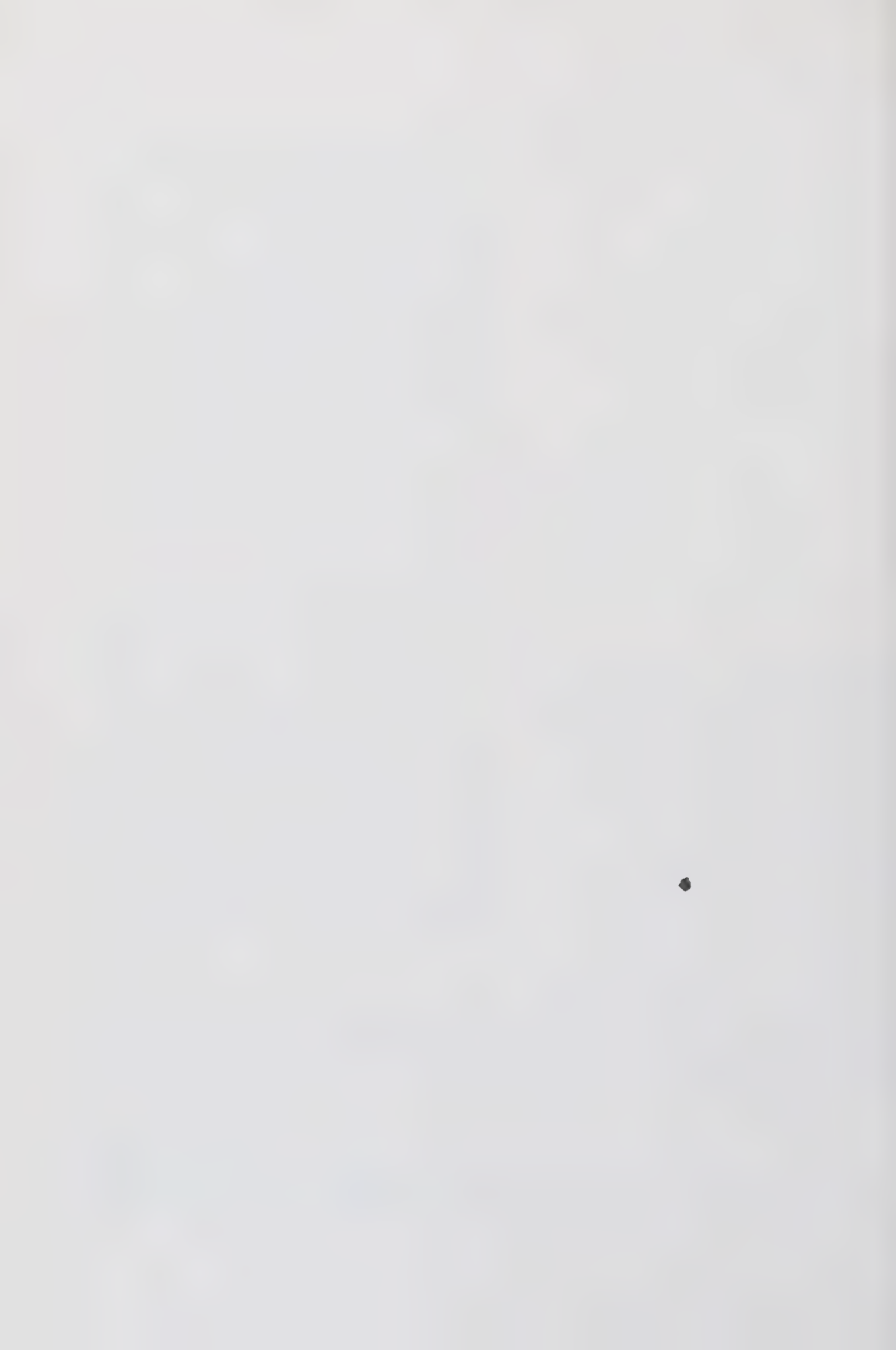
12 This figure has been tentatively interpreted as similar to the "neurotubules" described by De Robertis and Schmitt ('48a, b).

13 These elements probably represent the units of structure in the conductive fibers of Purkinje.

14 and 16 Small myofibrils displaying light and dark bands. In 16 a portion of the "wall" appears to have been lost leaving a central non-striated portion. This may be the result of a "clot."

15 Probably some form of connective tissue element. Small enlargements along the fiber are shown. These do not seem to correspond to the cross bands of collagen fibers.





THE GROWTH OF THE SPLEEN IN THE CHICKEN ¹

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TWO FIGURES

Recent years have seen a renewal of interest in the problem of the place of origin of antibodies. Lymphocytes (Enrich and Harris, '42; White and Dougherty, '46), plasma cells (Bjørneboe, Gormsen and Lindquist, '47) and the developmental stages of plasma cells (Fagraeus, '48) have been said to be associated with antibodies or antibody production. The reticulo-endothelial system in general (Sabin, '39) certainly seems to play a part in the production of these substances. The present paper deals with the growth of the spleen, an organ which is part of the reticulo-endothelial system and which is therefore probably associated with antibody production. This study correlates the changes in weight of the spleen with the age of the chicken from hatching to adult. The only comparable study previously made was done on the rat. Most of the research with other animals has compared the weight of the spleen with the total weight of the body of the animal regardless of age.

The correlation of spleen weight with body weight has been studied in the rat by Jackson ('13), Donaldson ('24), Reinhardt ('43) and Webster, Liljegren and Zimmer ('47). Reinhardt found that the spleen increased in the growing animal until about 100 days of age at which time the spleen attained its maximum size. Beyond 100 days a slight decrease in the

¹Supported in part by the Research Committee of the University of Wisconsin Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

spleen size was noted and this continued up to 225 days at which time the study was terminated.

Latimer ('24) made a study of the organ weight and growth of White Leghorn chickens. He autopsied 94 chicks of varying ages and 6 adults, and compared spleen size with body size. No correlation of spleen size with age was made. His graph showed that young chicks that weighed about 400 gm had the largest spleens relative to body weight (0.24% of the total body weight).

The relative size of the spleen is greater in mammals than in lower animals. In 300 gm rats the spleen constitutes about 0.25% of the body weight, while in 75 gm animals it is 0.50% of the body weight (Webster, Liljegren and Zimmer, '47). In man the spleen is about 0.4% of the total body weight (Perla and Marmorston, '35), while in Reptilia and Amphibia the spleen is about 0.06% (Klemperer, '38), and in Rhine salmon from 0.5 to 0.21% of the total body weight (Meischer, quoted by v. Skramlik, '27).

There is no difference in the relative size of the spleen correlated with sex in the rat (Webster, Liljegren and Zimmer, '47), but Bessenden and Carlson ('23), Eaton ('38) and Andreasen ('46) working with the guinea pig report a sex difference, the female guinea pigs having larger spleens in most strains. In the ring-dove Riddle ('28) finds that the spleen of the female exceeds the weight of that of the male by an average of 23.5%. Latimer ('40) reports that the spleen of the male cat is significantly heavier in absolute weight and is also more variable in weight than that of the female.

In many of the animals studied the coefficient of variation for body weight has been reported to be around 15% while that of the spleen is about 30%. The values reported for the guinea pig (Eaton, '38) are somewhat lower than these while those for the cat (Latimer, '40) are slightly higher.

MATERIALS AND METHODS

For the purposes of this study a total of 1023 chickens, mostly a White Leghorn-Barred Rock cross and a few White

Leghorns or New Hampshire breed, were obtained from the Poultry Husbandry Department of the College of Agriculture at the University of Wisconsin. The chickens ranged in age from one day to about one year. Most of the age groups studied were represented by about 40 chickens. There were about equal numbers of both sexes. The animals were killed by breaking the neck or by decapitation. Loss of blood with the latter method was not considered since in separate experiments no effect of bleeding on the weight of the spleen of the chicken could be found. Only chickens which appeared to be normal and healthy were used; sickly chickens or "runts" were discarded. All chickens were examined especially for evidence of coccidiosis or other disease. The animals were weighed and killed and the spleens were removed at once and weighed in a closed balance to prevent loss of water. Care was taken to remove all excess fat and mesentery from around the spleen before weighing.

The chickens were received at different times over a period of months. Not all the chickens in each age group were obtained at the same time. The animals were received at different ages and were kept from one to several days under as constant conditions as possible. The effect on the chickens of possible variables in the conditions will be discussed later.

RESULTS

The results for all age groups are summarized in table 1. Of the 1023 chickens studied, 465 were males and 558 females. The chickens are divided into age groups, each group containing all chickens of an age not more than 4 days or less than three days from the designated age of the group. For example, all chickens between the ages of 5 weeks and 5 days, and 6 weeks and 4 days are included in the group denoted as 6-week old chickens. The first column of the table gives the age group, the second column the number of males and females in that group. The body weight is given in grams and the spleen weight in milligrams plus or minus the standard error (or standard deviation of the mean). The coefficients of varia-

tion of the body weights and spleen weights are expressed as per cent. In the last column of the table the spleen weights are expressed as per cents of the total body weights for each age group.

TABLE 1
Body and spleen weights of growing chickens

AGE	SEX AND NO. OF CHICKENS	BODY WT. (GM) ± STANDARD ERROR	BODY WT. COEFFICIENT OF VARIATION	SPLEEN WT. (MG) ± STANDARD ERROR	SPLEEN WT. COEFFICIENT OF VARIATION	SPLEEN WT. AS PERCENTAGE BODY WT.
<i>days</i>			<i>%</i>		<i>%</i>	
1 and 2	♂ 30	32 ± 0.58	10.6	11 ± 0.44	21.8	0.033
	♀ 31	32 ± 0.50	8.7	10 ± 0.41	23.0	0.031
<i>weeks</i>						
1	♂ 55	50 ± 1.14	16.9	33 ± 1.71	25.2	0.066
	♀ 48	51 ± 1.24	16.8	36 ± 2.27	30.8	0.070
2	♂ 49	60 ± 2.45	28.6	52 ± 5.87	79.0	0.088
	♀ 73	61 ± 1.85	25.8	58 ± 3.91	57.6	0.096
3	♂ 26	105 ± 3.95	19.1	118 ± 12.6	54.6	0.110
	♀ 41	98 ± 4.51	29.6	111 ± 10.2	59.1	0.113
4	♂ 27	134 ± 7.50	29.1	149 ± 10.1	35.2	0.111
	♀ 51	155 ± 5.89	27.1	213 ± 11.4	38.4	0.138
5	♂ 67	175 ± 8.09	37.8	222 ± 16.6	61.2	0.127
	♀ 77	193 ± 7.34	33.4	296 ± 17.9	53.0	0.153
6	♂ 31	350 ± 18.9	30.0	503 ± 31.4	34.8	0.143
	♀ 42	326 ± 16.6	32.8	546 ± 37.7	44.7	0.167
7	♂ 52	375 ± 23.2	44.5	560 ± 51.0	65.6	0.149
	♀ 50	379 ± 18.9	35.1	701 ± 49.2	49.6	0.185
8	♂ 30	453 ± 30.3	36.6	697 ± 54.4	42.7	0.154
	♀ 29	444 ± 32.1	38.7	816 ± 81.5	53.8	0.184
9	♂ 6	642 ± 20.2	7.7	1199 ± 114	23.3	0.187
	♀ 11	617 ± 15.5	8.3	1320 ± 79.7	20.0	0.214
10	♂ 16	808 ± 42.1	20.8	1509 ± 91.5	24.2	0.187
	♀ 21	789 ± 26.9	15.6	1851 ± 140	34.6	0.234
11	♂ 32	771 ± 19.3	14.1	1424 ± 68.3	27.1	0.185
	♀ 11	621 ± 31.4	16.7	1118 ± 88.5	26.3	0.180
12	♂ 19	922 ± 41.9	19.9	1688 ± 125	32.4	0.183
	♀ 24	882 ± 34.2	19.1	1769 ± 135	37.3	0.201
13	♂ 15	1068 ± 47.3	17.2	1901 ± 95.0	19.2	0.178
	♀ 28	853 ± 22.7	14.1	1501 ± 66.4	23.4	0.176
14	♂ 3	975 ± 50.2	8.9	1510 ± 55.1	6.3	0.155
16	♂ 7	1714 ± 52.0	8.0	2912 ± 154	14.0	0.170
	♀ 2	1293 ± 33.5	3.7	2634 ± 266	14.3	0.204
1 yr.	♀ 19	1815 ± 64.2	15.4	1890 ± 133	30.6	0.104

The standard errors and coefficients of variation for the spleen weights are larger than those for the total body weights, showing that the spleen is more variable in size than the body as a whole. This correlates with the results of other investigators on other animals. About the same amount of variability was observed in both sexes. The coefficients of variation of both the body weights and spleen weights are high compared to reported values for some other animals. The coefficients of variation in this study average about 23% for the body weights and about 39% for the spleen weights. Several factors may be mentioned as contributing to the variation observed in both the body and spleen weights. In some of the age groups the chickens were hatched at very different times of the year and were therefore subjected to differences in conditions affecting growth, such as temperature, even though all birds were raised indoors. Feeding of the chickens was constant after they were received but there may have been some variation in their treatment prior to this. An example of the result of some of these variables is to be found in the data for 7 weeks of age. This group is composed of chicks from 5 hatchings, December 19, January 13, January 16, March 10, and August 13. The chicks from the first two hatchings were much lighter in weight than the last three hatchings. This raises the standard error and coefficient of variation above what it would be for each hatching considered separately. However, the average size and total variation from all hatchings were felt to be of most importance. The data for separate hatchings were therefore pooled.

The data were considered for a possible association of body and spleen size with sex. Only the data for the first 13 weeks of age were used, the information on the older groups being incomplete. No marked sex difference in weight is found in chicks up to 13 weeks of age. The data in table 1 indicate that beginning with the 8th week, the average body weight of the males is larger than that of the females, and that from about this time the males grow at an increasingly more rapid rate than the females. At 8 weeks the average

body weight of the female is 98% of the male; at 13 weeks it is only 80%. This increase is not great enough to result in a statistically significant difference in the body weights for the first 13 weeks (calculated $t=1.83$; $t_{0.05}=1.96$).

No significant difference in the spleen weights of males and females during the first 13 weeks was found (calculated $t=0.51$). Since there was a difference in the body weights of males and females, although not a significant one, the data were adjusted by taking into consideration the slightly heavier body weights of the males. The males were found to have an average body weight 1.08 times heavier than that of the females. The spleen weights of the females were accordingly adjusted to the heavier body weights of the males by multiplying them by 1.08. These adjusted figures show that the females have significantly heavier spleens than the males (calculated $t=2.02$).

The last column in table 1 records the spleen as a per cent of the total body weight and this gives some measure of the rates of growth of the spleen as compared to the body as a whole. The spleen reaches its maximum per cent of the body weight at about 10 weeks. Up until this time the spleen has grown more rapidly than the rest of the body. After about 10 weeks the spleen slows down and the body grows relatively more rapidly. There are indications that a slight decrease in the size of the spleen takes place in chickens over one year old.

In figure 1 the growth curves for the spleen and body weight are shown. The curves rapidly diverge with the spleen growing at a faster rate than the body as a whole. The period of the most rapid growth of both the spleen and body weight is from about the 7th to the 10th week of age. After this both continue to grow at a somewhat slower rate, although from this time on the body weight is increasing slightly faster than the spleen weight.

In order to compare the growth of the spleen with that of another organ in the body, the testes of some of the chickens were weighed. The average weights of the testes for these birds are plotted in figure 1. A very different picture from

that of the spleen is seen. The testes grow very little until about the 11th weeks of age at which time they enlarge rapidly. During this period the spleen has already passed its maximum rate of growth.

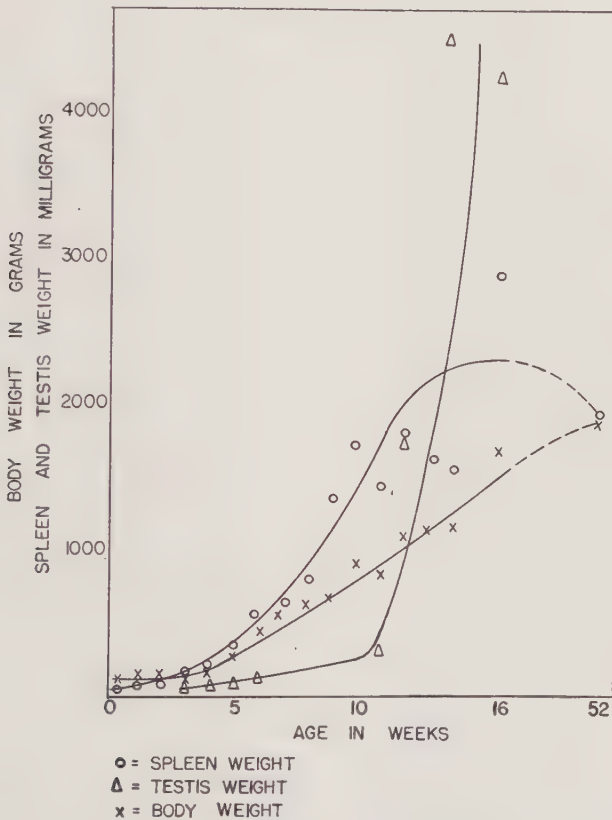


Fig. 1. The changes in spleen, testis and body weight in the growing chicken. The average weights of spleen, testis and body are graphed for the age groups in table 1. The curves are not calculated but are drawn in for greater clarity.

In figure 2 the weight of the spleen expressed in per cent of the total body weight is plotted for each age group. The graph shows a period of rapid relative growth of the spleen from hatching until 7 to 10 weeks of age. After this age the relative weight of the spleen decreases, showing that the

body from this time on is growing at a more rapid rate than the spleen. Some of the later points on the curve are insufficiently established but the general trend of relative decrease in spleen size is unmistakable. This decrease in size relative

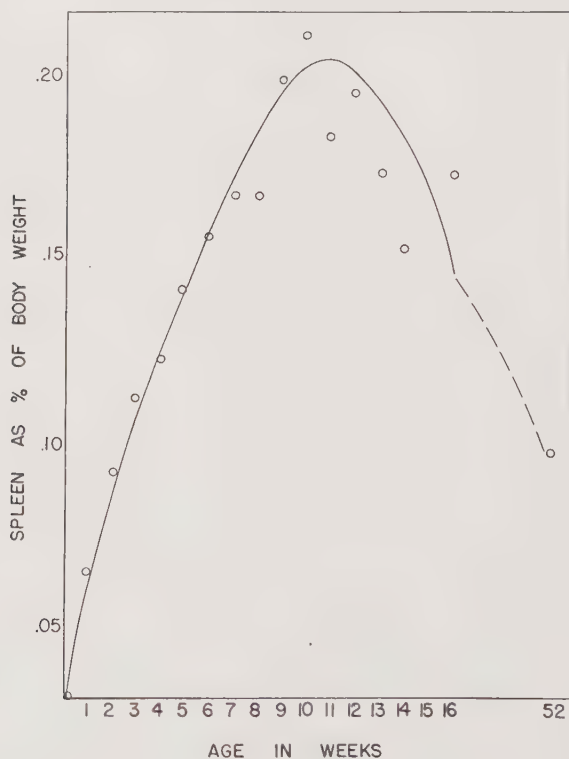


Fig. 2. The changes in the spleen weight expressed as a per cent of the body weight in the growing chicken.

Each point represents the average per cent weight for the male and female chickens for that age. The curve is not calculated but is drawn in for greater clarity.

to the body size could be due to an acceleration of the rate of body growth without a slowing down of the rate of growth of the spleen. That this is not the cause of the decrease in relative size of the spleen was shown graphically in figure 1. After 10 weeks of age the slope of the curve of body growth

is not increased and the curve may even be somewhat flatter than before. Therefore the decrease in the spleen weight relative to body weight noted in figure 2 must be due to decrease in growth rate of the spleen.

DISCUSSION AND CONCLUSIONS

The spleen has been shown to enlarge at a rapid rate in the growing chick. This condition holds until about the 10th week after hatching. In the first 6 weeks after hatching the spleen undergoes about a 50-fold increase in size. During the same period the body increases about 10-fold. Thus the rate of growth of the spleen is much greater than that of the body during that time. From 6 to 12 weeks the spleen about triples in weight as does the body weight also. After this period little, if any, increase in the size of the spleen is noted. The body, however, continues to grow for some time.

The rapid growth of the spleen in the immature chicken is of interest in connection with precipitin production. It has been shown by Wolfe and Dilks ('48) that the newly hatched chick is a very poor precipitin producer, but that this ability increases gradually until 5 weeks of age and then a sudden spurt occurs. Serological "maturity" based on the tests used, is reached at about 5 or 6 weeks of age. The spleen has been shown to be related to antibody production and a certain stage of maturity of the spleen, as indicated here by the attainment of a certain size, may be necessary before precipitins can be produced in the chicken.

SUMMARY

1. The spleen of the chicken was found to be more variable than the body weight as expressed in standard error and coefficient of variation.

2. When corrected for the heavier body weight of the male chickens the spleens of female chickens were found to be significantly heavier than those of males.

3. The maximum size of the spleen was attained at three to four months of age.

4. A suggestion is made that it may be necessary for the spleen to reach a certain stage of growth before it becomes "serologically mature".

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CYTOLOGIC STUDIES OF THE REMAINING KIDNEY FOLLOWING UNILATERAL NEPHRECTOMY IN THE RAT

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FIFTEEN FIGURES

INTRODUCTION

Although compensatory renal enlargement has been extensively studied in the albino rat, several important points especially concerning the nature of the reparative process remain unsettled. More exact knowledge regarding these points is urgently needed since numerous physiological and endocrinological investigations have been reported recently in which the rate of enlargement of the remaining kidney following unilateral nephrectomy has been used as a measure of growth without any attempt to define the exact nature of the enlargement.

All investigators agree that unilateral nephrectomy is followed by an increase in the size and weight of the remaining kidney. Not all agree regarding the amount of increase which may take place. Jackson and Shiels ('27) reported that after 7 to 101 days following unilateral nephrectomy in albino rats the weight of the remaining kidney was 34 to 134% (average 89%) above normal. According to Addis and Lew ('40) the weight of the remaining kidney was increased 70% in 40 days. The rate of restoration is at first rapid, but quickly decelerates and ceases completely about 40 days after removal of the other kidney. Arataki ('26) reported weight increases in the ratio of 1:1.34 to 1:1.80. Morel and Verliac ('13) found

that 50 days following unilateral nephrectomy the remaining kidney has increased in weight 52 to 64%. Tuffier (1888) estimated that 20 to 25 days are required for the anatomic completion of a compensatory hypertrophy after nephrectomy, and that in this time, the weight of the remaining kidney is increased by one-fifth to one-sixth of its original weight.

The differences in the results reported may be due to variation in technics, adjustment for the normal expected increase in weight and differences in the ages of the animals. Hinman ('26) reported that the hypertrophy observed in the remaining kidney following unilateral nephrectomy in young and growing animals and in animals which were fully developed at the time of the operation varied markedly. Braun Menendez ('46), on the contrary, found no significant difference in the increase in weight of the remaining kidney which could be correlated with the ages of the animals.

Before 1900 some investigators (Tizzoni and Pisenti, 1883; Lorenz, 1886) believed that true renal regeneration is the mechanism involved in compensatory enlargement of the kidney following unilateral nephrectomy. They reported an increase in the number of glomeruli and the sprouting of new buds from existing tubules. Galeotti and Villa-Santa ('02) reported that there is a true hyperplasia of glomeruli and tubules following nephrectomy in young dogs and rabbits but that this does not occur in adult animals. All later reports indicate that there is no increase in the number of glomeruli and tubules except in the case of very young animals in which the development of the kidney is as yet incomplete.

Mitotic activity or hyperplasia of cells lining the tubules and the capsules following nephrectomy has been reported by Albarran (1899), Ribbert (1882), Eckhardt (1888), Fiori ('01) and Hinman ('26). This phenomenon has been neglected or denied by most of the other investigators. They regard the increase in size of the tubules and the glomeruli as hypertrophy. Lorenz (1885) and Galeotti and Villa-Santa ('02) reported no mitotic activity. Saphir ('27), studying experimental renal hypertrophy in rabbits reported that the en-

largement of the kidneys is due in part to hypertrophy of the glomeruli with no multiplication of cellular elements. He also pointed out that the lining cells of the convoluted tubules show marked cloudy swelling and that no mitoses were seen. This he stated is contrary to the observations of Hinman. He further reported that during the first three weeks of hypertrophy, the kidneys show first a hyperemia of the capillaries and later a marked cloudy swelling.

Jackson and Shiels ('27), whose observations began 7 days after nephrectomy, reported that the histological structure in general appears normal, and that there is certainly no excessive hyperemia, edema or other abnormal conditions which could account for the great enlargement. They concluded that the renal enlargement in their series was due chiefly to an increase in the amount of renal parenchyma, rather than to an increase in the tubular lumina, the blood content or the stroma. They did not determine whether the increase involves cellular hyperplasia, cellular hypertrophy, or both.

Allen and Mann ('35) reported that the most satisfactory working hypothesis relative to the cause of the phenomenon of compensatory renal hypertrophy is that it constitutes a physiologic or work hypertrophy. The solitary kidney responds by a gradual enlargement of its glandular elements, the glomeruli and tubules, which results finally in an increase in mass, or compensatory hypertrophy, of the kidney.

In recent investigation, Braun Menendez ('46) concluded, on the basis of histologic observations and the modification of the weight of the kidney dried by heat, that the early increase in weight is due principally to the accumulation of liquids.

Boyd ('47) compared the compensatory enlargement of the kidney with that of the liver and the thyroid. When a portion of one of the latter organs is removed the organ is restored to its original size. This he regarded as hyperplasia in contrast to the hypertrophy which occurs in the kidney.

He stated that hypertrophy in the kidney is most easily recognized in the convoluted tubules, in which the lining cells become larger and the lumina become dilated.

MATERIALS AND METHODS

Twenty-eight albino rats of the Wistar strain were used in this study. The animals were obtained from 6 litters and were approximately 90 days old at the initiation of the experiment.

The unilateral nephrectomies were carried out with the animals under ether anesthesia. A one-half inch incision was made along the edge of the lumbar muscles just caudal to the last rib. The kidney was exposed, ligated with linen thread, and excised. The muscles and the integument were closed separately.

The contralateral kidneys were removed at intervals of 24 hours to 20 days following the initial operations.

The kidneys were sliced, fixed in Zenker's fluid, dehydrated and imbedded in the usual manner. Sections of the control kidney of each animal were placed on the same slide with sections of the contralateral experimental kidney and were stained with Harris hematoxylin, by the Feulgen method or with phloxine-methylene-blue. For the sake of uniformity all measurements of nuclei were made from material stained by the Feulgen method.

HISTOLOGIC DATA

Mitotically active cells in the tubular epithelium were observed in at least 8 of the control kidneys in this series. Since the number of mitotic figures is very small, their occurrence in the other control kidneys is not precluded. In no instance were more than 4 dividing cells observed in a complete longitudinal section, 7 μ thick, of the normal kidney. This does not include cells which may have been in the early prophase stage.

Sections of kidneys that were removed 24 hours after contralateral nephrectomy contained few cells in active division.

They were more numerous in these sections, however, than in sections of a control kidney. In sections of kidneys removed 48 hours to 20 days after unilateral nephrectomy mitotic figures in the epithelia were very numerous. The peak of mitotic activity occurs between 72 hours and 240 hours following unilateral nephrectomy. During this period it was not uncommon to observe 4 or more dividing cells in a single oil immersion field (fig. 4). The mitotically dividing cells were found in the tubules of all of the types (figs. 4, 5 and 6) and as well as in the parietal (fig. 7) and the visceral layers (fig. 8) of Bowman's capsules. The distribution of dividing cells is not uniform throughout the renal parenchyma. Some areas appear to be active mitotically, while others are inactive. No definite pattern of mitotic activity could be recognized.

In most instances, the planes of division of epithelial cells are perpendicular to the axes of the tubules. In some instances they are parallel to the tubules. In the former instance, new cells are added to the circumference of the tubule and it assumes a greater diameter. In the latter instances, new cells are budded off into the lumen. These may become detached from the wall of the tubule, undergo further division and form epithelial plaques in the lumina. Such plaques were not observed in sufficient numbers to cause congestion in the renal tubules.

Sections of both control and experimental kidneys contain cells with more than one nucleus. Binucleate cells are common and are found in all the tubules as well as in the walls of the renal corpuscles. The results of counts of binucleate cells in the cortex and in medulla respectively in the control and in the experimental kidneys are plotted in figures 1 and 2. Counts were made in 100 fields selected at random in the cortex of each experimental kidney and its contralateral control as well as in the medulla of each pair. The counts in the cortex and in the medulla were made separately because there were more cells per field in the medulla. Counting 100 fields in the cortex and the medulla respectively revealed

that there were 45.17 ± 7.96 cells per field in the former and 81.70 ± 8.28 cells per field in the latter.

The results shown in figures 1 and 2 indicate that the number of binucleate cells in the various control kidneys is relatively constant and that there is no significant difference in the number of binucleate cells in the kidney 24 hours after removal of its mate. Preparations of all the kidneys removed

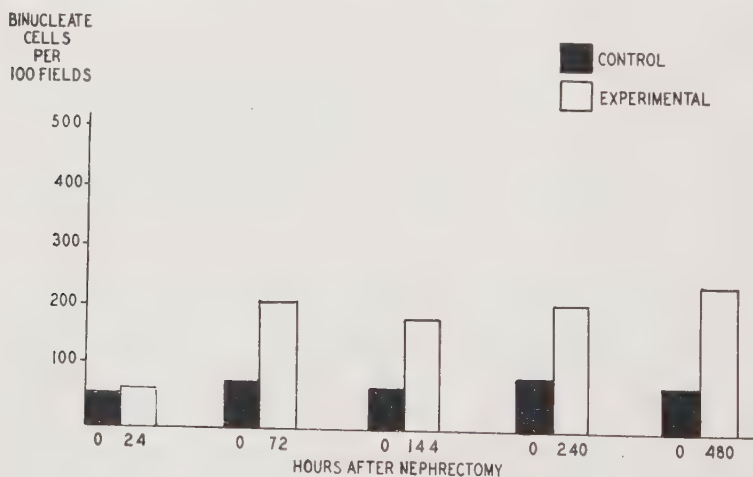


Fig. 1 The occurrence of binucleate cells in the cortex of the normal kidney and in the remaining kidney following nephrectomy.

later than 24 hours after unilateral nephrectomy show a significant increase in the number of binucleate cells. The increase appears to be much greater in the medulla than in the cortex.

Cells with more than two nuclei were observed in most of the experimental kidneys. They included cells with three to at least 6 nuclei (fig. 10). In some instances two mitotic figures with separate spindles occurred in the same cell (fig. 11).

Variation in the sizes of nuclei even within the same tubule were noted in sections of the control and the experimental kidneys (fig. 12). For the sake of uniformity, measurements were made only of the nuclei in the proximal convoluted tu-

bules in a normal kidney and in the contralateral kidney which had been allowed to remain for 20 days following the removal of the control. Drawings were made at the table level at a magnification of approximately 1900 diameters with the aid of a camera lucida. The drawings of the nuclei were measured to the nearest $\frac{1}{2}$ mm at their smallest and largest diameters and the measurements were averaged. The few examples in

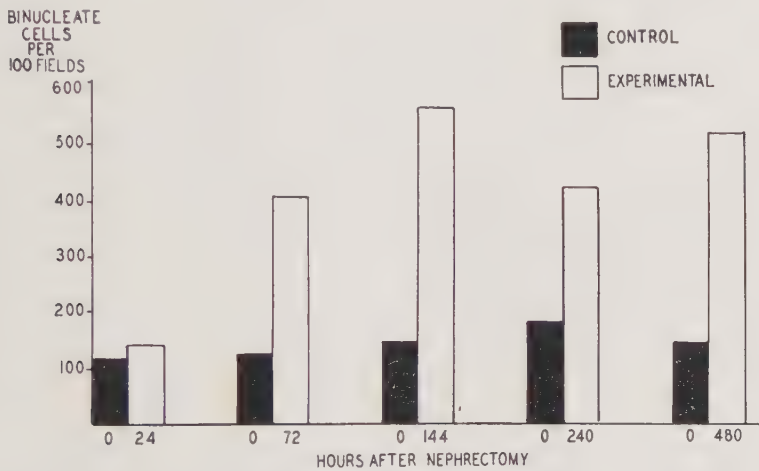


Fig. 2 The occurrence of binucleate cells in the medulla of the normal kidney and in the remaining kidney following nephrectomy.

which the difference in dimensions exceeded 2 mm were discarded.

Three hundred nuclei of cells in the proximal convoluted tubules in control and experimental kidney were chosen at random and measured in the manner described above. The diameter in millimeters was plotted against frequency and the results are presented in figure 3. These curves indicate that in the normal kidney the nuclei, with respect to their linear dimensions, fall into three modes, with peaks at 18, 23, and 28 respectively. The last mode would be insignificant were it not for the fact that it is prominent in the experimental kidney. In addition to the aforementioned modes there appears to be a 4th mode in the experimental kidneys with a

peak at 33. The nuclei being spherical, the cube of their diameters will give their comparative volumes. The volumes at the peaks of the nodes fall closely into the ratio of 1:2:4:8. This indicates that there are three classes of nuclei in the proximal convoluted tubules in the normal kidney. Most of the nuclei fall into the first class, a relatively small number into the third class. In the experimental kidney the percentage of nuclei which fall into the second two classes are much higher than in the control kidney. These data indicate that polyploidy exists in the tubular epithelium of the normal kidney

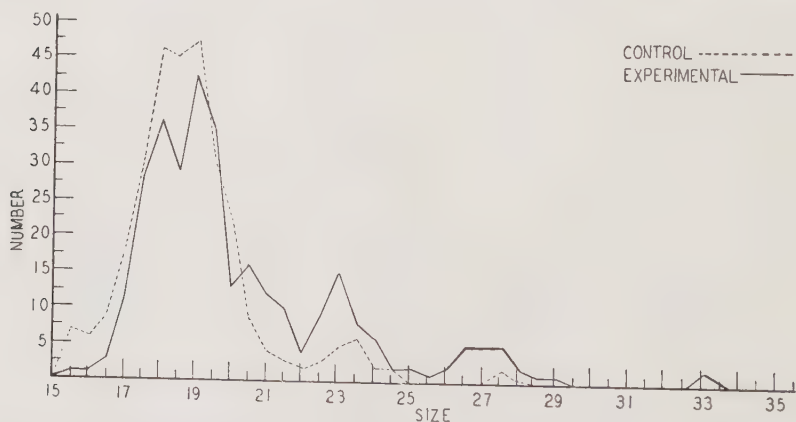


Fig. 3 Distribution of nuclei of the proximal convoluted tubules arranged according to linear dimensions in the normal kidney and in the remaining kidney 20 days following unilateral nephrectomy.

and occurs with greater frequency and magnitude in the remaining kidney following nephrectomy. Although it is not feasible to count the chromosomes, difference in chromatin mass may be observed in diploid nuclei (fig. 13) and in polyploid nuclei (fig. 14).

DISCUSSION

The role of the karyokinetic process appears curiously to have received minor consideration as an important factor in renal compensatory enlargement. In the normal kidney, the occurrence of mitotic activity is not entirely unexpected, especially in animals which have not reached senility. It has

been shown by Walter and Addis ('39) that in the kidney the general ratio of growth is at all times the same relative to the growth of the body. The mitotic activity in the normal kidney can therefore be explained as the process of normal growth. In renal compensation there is a sharp increase in mitotic activity. The peak of the mitotic activity was found to occur between three days and 10 days following unilateral nephrectomy. This parallels the observations made by Morel and Verliac ('13) who found that from 4 to 10 days following unilateral nephrectomy the remaining kidney increased from 32 to 37% percent in weight, from 10 to 20 days, from 9 to 16%, and from 20 to 50 days from 11 to 16%.

Considering the total volume of the kidney, it can be roughly estimated that during a 24 hour period, for example 72 hours after unilateral nephrectomy, there are several million cells undergoing mitotic activity. This must be considered an important factor in renal enlargement.

The concept of multinucleate cells and polyploidy in the mammalian kidney appears to have been neglected. Dawson ('48) investigating the kidney of an Australian desert frog, *Cyclorana* (*Chiroleptes*) *alboguttatus* (Günther) reports that he found a variation in the number and size of nuclei in the cells of the kidney tubules. He regards the large nuclei as polyploids. Other authors have mentioned large cells with large nuclei (Jiménez Díaz, et al., '48; Thorel, '03; Oliver, '15; Lubarsch, '25) in renal epithelium. Some of these investigators who observed these cells in the kidneys that were recovering from toxicity consider them as cells of regeneration, although Lubarsch regards them as simply swollen cells.

There is some evidence that polyploidy in the renal parenchyma arises in the same manner as it has been reported to arise in the liver parenchyma by Beams and King ('42) and Sulkin ('43). According to Beams and King the mechanism involved in forming polyploid mononucleate cells is simply that during mitosis, the cytoplasm fails to divide, thus giving rise to a binucleate cell, with each nucleus possessing the usual diploid complement of chromatin. At the next mito-

tic division of the binucleate cell, the chromosomes of each nucleus merge in the metaphase plate, resulting at the completion of division in either (1) two large mononucleate (tetraploid) daughter cells or (2) one large binucleate cell with the octaploid chromatin complement (i.e., each nucleus tetraploid). A late prophase in division of a binucleate epithelial cell, with the chromosomes forming one equatorial plate is shown in figure 15. There is no indication that in the renal epithelium large nuclei may be formed by the fusion of smaller nuclei, a phenomenon which has been reported by Wilson and Leduc ('48) to occur in mouse liver, nor is there any evidence that some large nuclei are produced by endomitosis, as reported by Bieseke, Poyner and Painter, ('42) also in the liver. It may be pointed out that indications of amitotic division of the renal parenchyma were never observed.

Large multinucleated cells in the kidney have been reported by many investigators, the most recent being Jiménez Díaz, et al. They conclude, that in the kidneys recovering from uranium intoxication, these cells divide and form islets of new cells or pseudotubules. These are usually located in the inner lining of the tubule where they may protrude into the lumen and close it up. It is possible that earlier workers considered these plaques to be regenerating tubules.

The relative extent to which cortex and medulla are involved in compensatory renal enlargement has been studied by Ribbert (1882) in young puppies and rabbits, by Peruzzi ('15) in newborn rabbits, and by Bayle ('26) in adult rabbits. These investigators have found the cortex to be the part chiefly or entirely concerned in the enlargement. Arataki ('26), on the other hand, found the medulla:cortex ratio not much changed. Jackson and Shiels ('27) conclude that the compensatory enlargement, although chiefly affecting the cortex, must also involve the medulla. The data reported here, concerning the distribution of the increase in binucleate cells tends to support the view of Arataki although there is not sufficient evidence to rule out the conclusions of Jackson and Shiels. The evidence, however, does not support the view

that the medulla is not involved in compensatory renal enlargement.

The present findings are not in agreement with the conclusions of Boyd ('47) who compares compensatory renal enlargement with compensatory hepatic enlargement, calling the former hypertrophy and the latter hyperplasia. It is suggested that the process in the kidney and in the liver are very similar, cellular proliferation and the formation of polyploid cells being involved in both instances, and in both instances being a hyperplastic process.

SUMMARY

Unilateral removal of a kidney in the rat is followed by compensatory enlargement of the remaining kidney. The nuclei in the cells of the various tubules in the normal and enlarging kidneys were studied. Mitotic figures are infrequent in the normal kidney, probably due to the slow rate of growth. In sections of kidneys removed 48 hours to 20 days after unilateral nephrectomy, mitotic figures in the epithelia were very numerous. The peak mitotic activity occurs between 72 hours and 240 hours following unilateral nephrectomy.

Binucleate cells are common in the normal kidney and in the kidneys undergoing compensatory enlargement. Results of counts indicate that the number of binucleate cells in the various control kidneys is relatively constant. Preparations of all the kidneys removed later than 24 hours after unilateral nephrectomy show a significant increase in the number of binucleate cells. Measurement of nuclei in the proximal convoluted tubules of the control kidney and of the kidney remaining 20 days after nephrectomy, indicate that in the former the measurements fall into three modes and, in the latter, into 4 modes. The volumes at the peaks of the modes fall into the ratio of 1:2:4:8. There is an increase in the frequency and magnitude of large nuclei in the experimental kidneys.

The data presented have been discussed in relation to compensatory renal enlargement.

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PLATE 1

EXPLANATION OF FIGURES

All figures are unretouched photographs taken with 10 $\times$ ocular (Zeiss Mobini) and 1.8 mm objective (Zeiss apochromatic).

4 Section of cortical portion of kidney showing 4 mitotic figures, two of them in one tubule, in a single oil immersion field. Feulgen-light green stain. $\times 900$.

5 Typical mitotic figure in collecting tubule, Feulgen-light green stain. $\times 900$.

6 Mitotic figure in cell of proximal convoluted tubule, Feulgen-light green stain. $\times 900$.

7 Mitotic figure in parietal wall of Bowman's Capsule, Feulgen-light green stain. $\times 900$.

8 Mitotic figures in visceral wall of Bowman's Capsule, Feulgen-light green stain. $\times 900$.

9 Cell with three nuclei in wall of tubule, Feulgen-light green stain. $\times 900$.

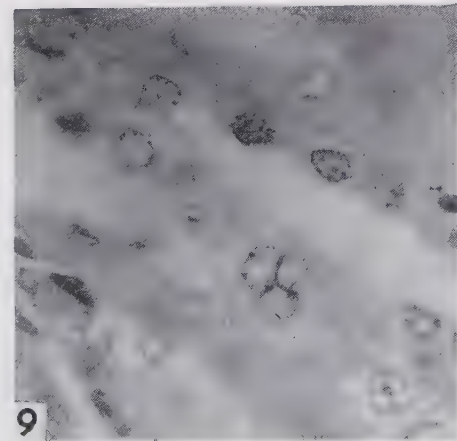
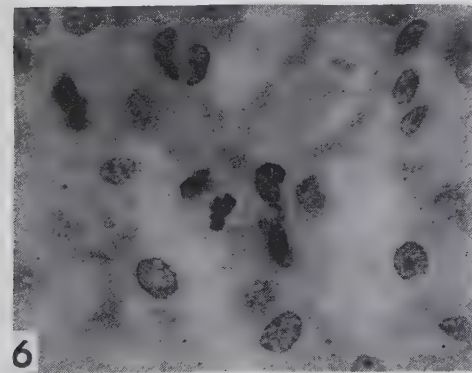
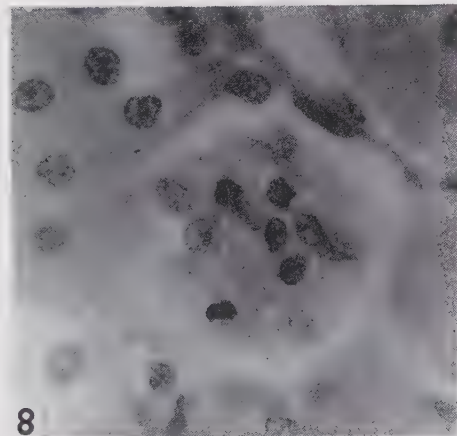
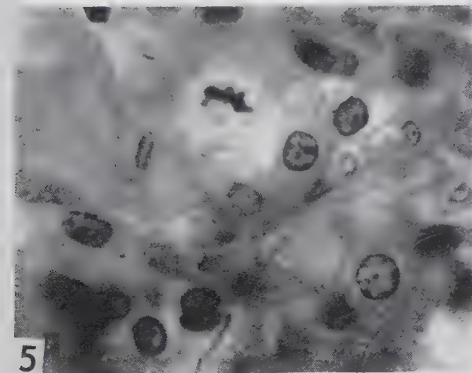
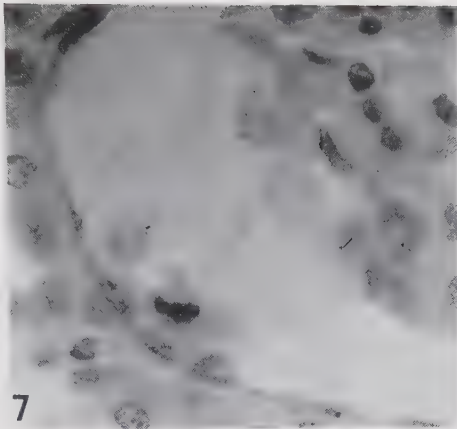
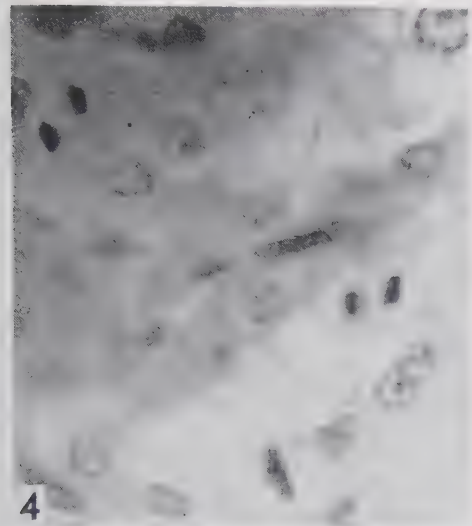
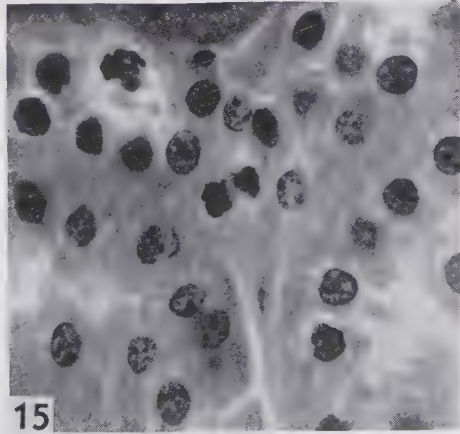
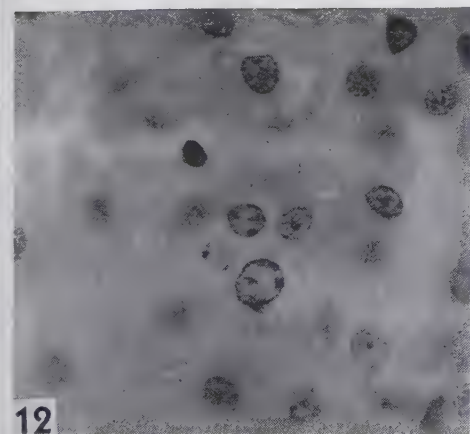
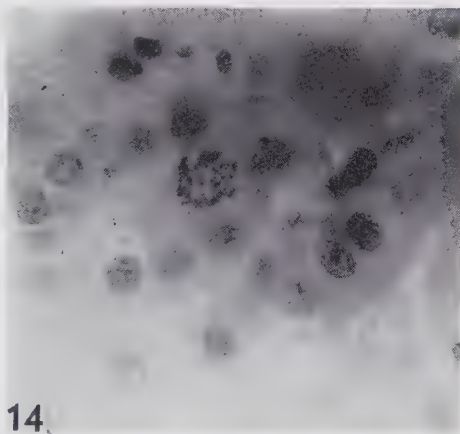
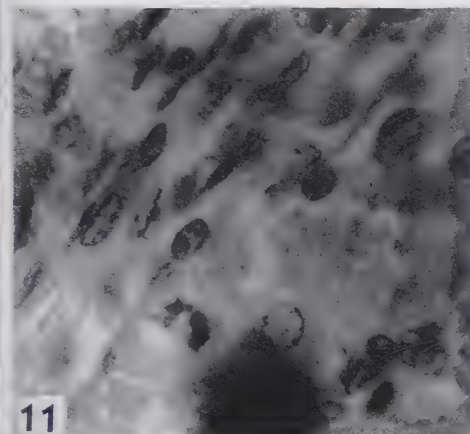
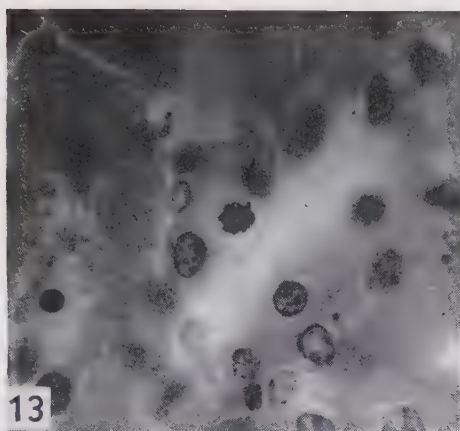
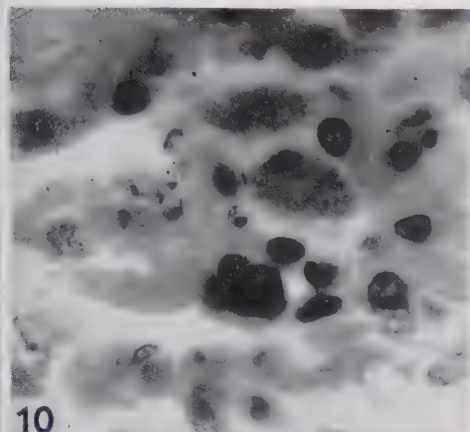


PLATE 2

EXPLANATION OF FIGURES

All figures are unretouched photographs taken with 10 $\times$ ocular (Zeiss Mobini) and 1.8 mm objective (Zeiss apochromatic).

- 10 Cell with 6 nuclei in wall of tubule, Harris hematoxylin-eosin stain. $\times 900$.
- 11 Two mitotic figures with separate spindles in one cell, Harris hematoxylin-eosin stain. $\times 900$.
- 12 Large nucleus and several small nuclei located in same tubule, Feulgen-light green stain. $\times 900$.
- 13 Mitotic figure with small eosin chromatin mass, Harris hematoxylin-eosin stain. $\times 900$.
- 14 Mitotic figure with large chromatin mass. Harris hematoxylin-eosin stain. $\times 900$.
- 15 Late prophase in division of a binucleate cell with chromosomes forming one equatorial plate, Harris hematoxylin-eosin stain. $\times 900$.



PROCEEDINGS
OF THE
AMERICAN ASSOCIATION OF ANATOMISTS
Sixty-second Annual Meeting

Notice of 1950 Meeting

THE NEXT ANNUAL MEETING
OF THE AMERICAN ASSOCIATION
OF ANATOMISTS WILL BE HELD
IN NEW ORLEANS,
WEDNESDAY, THURSDAY, AND FRIDAY,
APRIL 5, 6, AND 7, 1950,
AT THE INVITATION OF
THE LOUISIANA STATE UNIVERSITY
SCHOOL OF MEDICINE.
THE HEADQUARTERS HOTEL
WILL BE THE JUNG.

PROCEEDINGS OF THE AMERICAN ASSOCIATION
OF ANATOMISTS

SIXTY-SECOND MEETING

*Temple University, School of Medicine, Philadelphia, Pennsylvania
April 13, 14, and 15, 1949*

The Association held its Sixty-second Meeting in Philadelphia, Pennsylvania, by invitation of the Temple University School of Medicine, Wednesday, April 13, Thursday, April 14, and Friday, April 15. Demonstrations, business and general sessions were held in the Medical School, motion picture sessions and social sessions at the Bellevue-Stratford Hotel. The Local Committee was composed of Andrew B. Adams, James S. Deakins, Donald L. Kimmel, Elizabeth K. Moyer, Ralph R. Tyson, Jean K. Weston, and John Franklin Huber, Chairman.

Registered Attendance:

	1949	1948		1949	1948
Association Members	362	310	Out of town	596	521
Non-members	347	255	Local	113	44
	709	565		709	565

Geographical Distribution of Registrants:

Alabama	5	Minnesota	12	Utah	2
Arizona	0	Mississippi	1	Vermont	1
Arkansas	2	Missouri	16	Virginia	11
California	10	Montana	0	Washington	5
Colorado	3	Nebraska	3	West Virginia	2
Connecticut	23	Nevada	0	Wisconsin	13
Delaware	0	New Hampshire	0	Wyoming	0
Dist. of Columbia	21	New Jersey	9	Georgia	9
Florida	0	Tennessee	7	Idaho	0
Michigan	33	Texas	6	Illinois	41

Geographical Distribution of Registrants: (continued)

Indiana	4	North Carolina . .	14	Belgium	2
Iowa	10	North Dakota . .	0	Canada	28
Kansas	9	Ohio	21	England	1
Kentucky	6	Oklahoma	3	Germany	2
Louisiana	11	Oregon	1	Holland	1
Maine	7	Pennsylvania . . .	145	Italy	1
Maryland	52	Rhode Island . . .	9	Lebanon	1
Massachusetts . .	25	South Carolina . .	6	Montevideo	1
New Mexico	0	South Dakota . . .	2	Pakistan	1
New York	110	Australia	1		

RÉSUMÉ OF THE SCIENTIFIC PROGRAM

The sixty-second meeting of the American Association of Anatomists was opened in general session by President Bartelmez at 10:00 A.M., April 13, in the Medical School Auditorium of Temple University. Nine papers were presented upon invitation of the Secretary. At the close of this session Dr. Bartelmez announced the appointment of Davenport Hooker and Jean Weston for the Auditing Committee for 1949.

The remainder of the program was divided into (a) Sessions for the reading of papers: Thursday morning, 9:00 to 12:15; Thursday afternoon, 2:00 to 5:15; and Friday morning 9:00 to 12:15; (b) a session of demonstrations Wednesday afternoon, 2:00 to 5:00; (c) a session for motion picture demonstrations Wednesday evening beginning at 7:15; and (d) a general session with an invitation program Friday afternoon 2:00 to 3:45. On the Thursday morning, Thursday afternoon and Friday morning sessions there were 6 concurrent programs.

The members of the local committee are to be congratulated on their arrangements for the program. Most of the rooms for scientific sessions had two entrances. There were adequate signs so that no one had any difficulty finding his way around. Not the least of the thoughtful devices of the local committee were the numerous chairs in the corridors so that visitors could rest between sessions.

Visitors who had not previously known the Medical School of Temple University came away from the meeting with a high opinion of the institution, its faculty, and its physical plant. The courtesies and helpfulness of everyone in the school contributed greatly to the smoothness of the meeting.

One of the features of the program which met almost unanimous favor was the establishment of set times for the papers presented from platform. We are indebted to Charles LeBlond for this valuable innovation.

NUMBER OF PAPERS AND DEMONSTRATIONS

	1949	1948	1947
Papers from platform	233	201	133
Papers withdrawn	5	5	2
Papers actually presented	228	196	131
Papers read by title	78	72	64
Demonstrations	66	45	51
Motion pictures	14	16	8

For the Friday afternoon invitational program, President Bartelmez invited two famous couples in Anatomy to present A Lewis and Clark Expedition in Anatomy:

WARREN H. LEWIS AND MARGARET R. LEWIS: The superficial gel layers of cells and eggs.

ELIOT R. CLARK AND ELEANOR LINTON CLARK: Histomechanical factors in the growth and behavior of blood vessels: (a) capillaries; (b) larger vessels, particularly arterial anastomoses, arteriovenous anastomoses, companion veins, and arterial coiling.

SOCIAL ACTIVITIES

Banquet: Attendance (410). Rose Garden, Bellevue-Stratford. Generous financial support of the W. B. Saunders Co. aided in lowering the cost of the tickets. After-dinner speaker: Marion Hines, Emory University, Atlanta, Georgia. Dr. Hines' address is printed in full at the end of the proceedings.

Smoker: Approximately 600. Ballroom, Bellevue-Stratford. Courtesy of The Blakiston Company.

Buffet Supper: Attendance (451). Ballroom, Bellevue-Stratford. Courtesy of the J. B. Lippincott Company.

Information Reception: Attendance (410). Oak Room and South Garden, Bellevue-Stratford. Courtesy of Lea & Febiger.

Tour of Historic Philadelphia: Attendance (40). Thursday, April 14, courtesy The Blakiston Company. Places visited: Congress Hall, Betsy Ross House, Old Swede's Church, Friends Meeting House, Independence Hall, Powell House, Christ Church.

MINUTES OF THE FIRST BUSINESS SESSION

April 14, 1949, 12:30 P.M.

President Bartelmez announced the appointment of the following committee; Nominating Committee for 1950: Horace W. Magoun, William U. Gardner, Allan L. Graffin, William W. Greulich, Walter E. Sullivan, Chairman.

The President reported that the minutes of the Sixty-first Session were printed in *The Anatomical Record*, v. 101, no. 3, July 1948, reprints of which had been distributed to all members, and would not be read unless called for. On motion duly made and seconded, the minutes were approved as printed.

Report of the Treasurer: The Secretary-Treasurer made the following report for the year 1948:

Financial Statement for the Year 1948

Balance on hand in checking account, December 31, 1947, in	
The Cleveland Trust Company, Cleveland, Ohio	\$2,630.04
Receipts from dues	2,414.59
Gift from Local Committee at Madison, Wis.	34.00
Total Credits	
Expenditures	\$5,078.63
Postage, supplies and miscellaneous	85.13
Wistar Institute, Philadelphia, Pa.	
Preliminary notices	122.17
Abstracts	594.57
Programs	397.61
Proceedings	231.62

Madison Meeting Expense	500.00	
(Paid to Local Committee)		
Secretary, travelling expenses	34.90	
Enrollment		
National Society Medical Research	100.00	
National Research Council Dues A. I. B. S.	676.50	
Cleveland Trust Company, Cleveland, Ohio		
Checks returned and processing charges	20.65	
Bank charge for handling account	5.24	
Total Expenditures	2,768.39	
		\$2,310.24
Balance on hand December 31, 1948, and deposited in the name of the American Association of Anatomists in the Cleveland Trust Company, Cleveland, Ohio		\$2,310.24
War Bond Defense Series F dated May 1942 in safe de- posit box, Central National Bank, Cleveland, Ohio.		
Value December 31, 1948	809.00	
Total Assets	\$3,119.24	

ANATOMICAL JOURNAL TRUST FUND

1948

Bonds on hand December 31, 1948		\$17,000.00
Cash on hand Central National Bank, Cleveland, Ohio, December 31, 1947	138.05	
Interest received from bonds during 1948	491.25	629.30
		\$17,629.30

No expenditures were made from this fund during 1948.

Report of the Auditing Committee: Dr. Hooker read the following report:

“We have examined the accounts of the Treasurer of the Association, for the calendar year 1948, have checked the receipts and expenditures by checkbook and bank statements and find these accounts correct.

We have also checked the receipts of the Anatomical Trust Fund for the same year, there having been no expenditures, and have found these accounts correct.”

(Signed) J. K. WESTON

DAVENPORT HOOKER

On motion made and seconded the reports of the Treasurer and of the Auditing Committee were accepted and adopted.

Election of Officers: The Nominating Committee, consisting of Olof Larsell, Chairman, Carl C. Speidel and Barry J. Anson, placed the following nominations before the Association: For members of the Executive Committee for terms expiring in 1953, James W. Papez and Gordon H. Scott.

In the absence of additional nominations, a motion was made, seconded and carried that the Secretary cast a unanimous ballot for the above-named nominees, and they were declared elected.

Election of Representatives and Committee Members: The Secretary presented, in behalf of the Executive Committee, the following nominations: Representatives to the meeting of the American Association for the Advancement of Science in New York, December 1949, James B. Hamilton and Joseph C. Hinsey. Representative on the Commission for Standardization of Biological Stains, Harold Davenport. Motion Picture Committee, W. H. Lewis, C. C. Speidel, E. R. Clark, Chairman. On motion of Dr. Clark, Joseph E. Markee was also nominated.

In the absence of other nominations, the above nominees were duly elected.

Membership report: The Secretary presented in brief the following report:

On June 1, 1948, the Association had 885 members. This number has been reduced during the year by 9 deaths and 5 resignations, two members to be dropped for non-payment of dues. (At present, 34 members are in arrears in payment of dues ranging from one to three years.)

Members deceased: The Association has suffered very great loss through the recent deaths of the following 9 members: Jules Duesberg, Hans O. Haterius, Otto Marburg, Robert O. Moody, Adolph R. Ringoen, Joseph L. Schwind,

G. L. Streeter, Harold L. Weatherford, and Franz Weidenreich.

HANS O. HATERIUS, professor and head of the Department of Physiology at the Boston University Medical College, died following surgery on June 28, 1948. Dr. Haterius was born in San Francisco, February 8, 1902. Elected to membership in 1933. Dr. W. R. Ingram has submitted the following biography of Dr. Haterius.

Dr. Haterius received his baccalaureate degree from Augustana College in 1923. His graduate training leading to the M. S. in 1926 and Ph.D. in 1928 was received in the Department of Zoology at the State University of Iowa. From 1928 to 1935 he was a member of the Department of Biology at New York University. In 1935 he accepted an appointment as assistant professor of physiology in the College of Medicine of the Ohio State University. His success as a teacher and investigator resulted in an appointment in physiology at Wayne University in 1938, where he became professor and head of the Department of Physiology in 1941. He accepted a call to the Boston University Medical College in 1945; here he served as professor and head of physiology until his death. Dr. Haterius' publications include fundamental contributions to the physiology of reproduction, of the anterior and posterior pituitary glands, and the mechanism of parturition. Recently he had been concerned with certain aspects of water diuresis and with effects of hypothermia. As a teacher Dr. Haterius was unusually successful and he found a high place in the affections of his students. Besides a membership in the American Association of Anatomists he was affiliated with the American Physiological Society, the American Society of Zoologists, Society for the Study of Internal Secretions, and Harvey Society, and the Society for Experimental Biology and Medicine.

OTTO MARBURG, born May 25, 1874, died June 13, 1948. Clinical Professor of Neurology, Columbia University College of Physicians and Surgeons. Elected to membership in

1946. Dr. Fred A. Mettler has submitted the following biography of Dr. Marburg.

Professor Marburg, born in Moravia, received his doctorate in medicine from the University of Vienna in 1899. From 1900-1903 and from 1906-1919 he served as an Assistant in the Neurological Institute of Vienna. Meanwhile he served in the clinics of Fuchs and Wagner—Jauregg and with Oppenheim in Berlin. In 1919 he succeeded his mentor, Obersteiner, as Director of the Neurological Institute of Vienna where he remained, pursuing the study of neuroanatomy and neuropathology, until his migration to New York in 1938.

Sponsored by the Rockefeller Foundation, he was able to pursue his fields of interest in the Department of Neurology of the College of Physicians and Surgeons at Columbia University where he held the post of Clinical Professor of Neurology. Although fatally ill, for some months prior to his death, he continued his microscopic studies until weakness ultimately made it impossible for him to attend the meetings of the Department and to visit his laboratory. Indefatigable industry and devotion to his field were Marburg's outstanding characteristics. Before me are three huge packets of reprints of his articles—only a portion of his production. He is perhaps best known for his *Mikroskopisch—topographischer Atlas des menschlichen Zentralnervensystem* (Leipzig and Wien. Deuticke, 1904) the third edition of which was published in 1927 and whose illustrations were characterized, as the *Revue de L'Universite de Bruxelles* put it, by "admirable finesse de dessin." He was also the continuing genius of Obersteiner's *Arbeiten aus dem Neurologischen Institute and der Wiener Universitat*. With Alexander he was co-author of the authoritative *Handbuch der Neurologie des Ohres* (Berlin, 1930).

The driving forces behind Marburg's devotion to his work were tremendous enthusiasm, which enabled him to bear his advanced age lightly, coupled with implicit faith in the dignity of scientific pursuits. He was resilient and possessed a sense of humor. He was frank and still profoundly generous

and kindly. He was not an actively religious man but his fundamental piety was at once obvious to all who knew him.

At the time of his death he was laboring on a most ambitious program—an atlas of neuropathology.

ROBERT ORTON MOODY died in Berkeley, California, on July 28, 1948 at the age of 83. Moody received his B.S. at Cornell in 1891 and his M.D. at Yale in 1894. He instructed in anatomy at Yale and Cornell and then in 1901 joined the Anatomy Department of the University of California where he rose to the rank of professor in 1932. He retired as emeritus professor in 1935. Dr. Moody was a member of the American Association of Anatomists from 1901 until his death.

Dr. Moody's best known research centered about the normal position of the viscera as revealed by the x-ray. This work, done at a time when any position of the viscera deviating from that in the cadaver was considered to be abnormal, significantly aided in changing this static concept. Moody was a devoted teacher and had broad interests in community health problems and in civic betterment.

The above biography was submitted by Professor P. E. Smith, who had been a colleague of Dr. Moody at the University of California.

ADOLF R. RINGOEN was born in Ridgeway, Iowa, January 1, 1887, died January 13, 1949. He received the A.B. degree from the University of Iowa, 1909; an A.M. degree from the University of Minnesota, 1913; and the Ph.D. degree from the University of Minnesota, 1919. Nearly all of his scientific career was spent at the University of Minnesota with the exception of one year which he spent as a fellow at Harvard. He rose through the ranks to the professorship of zoology. His chief interests were in embryology, histology, and endocrinology.

JOSEPH LOUIS SCHWIND, born 1902, died 1948. Francis Brun-
ning Professor of Anatomy and Chairman of the Department,
College of Medicine, University of Cincinnati. Elected to mem-
bership 1932. Dr. L. O. Morgan has submitted the following
biography.

Dr. Schwind was born at Dayton, Ohio, on July 12, 1902, and received his arts and masters degrees from Cornell University in 1924 and 1925 and his doctorate from Yale in 1928. Dr. Schwind's entire adult life was devoted to teaching, at which, as we know, he became a master. Beginning in his student years, he instructed in histology and embryology and rose in the hierarchy while teaching successively at Cornell, Yale, Georgetown, and Albany. His career culminated with his appointment to The Chair of Anatomy at the University of Cincinnati, College of Medicine in 1946. Dr. Schwind's investigative interest had been particularly embryological, later his studies concerned the blood and particularly the bone marrow.

In the two short years that we knew Dr. Schwind, he demonstrated his great influence with students. His gentleness and urbanity were to them as to us attractive as much as his learning and wit. Inquiry among students who had the opportunity of working with him elicits the fact that he was among the best teachers that they had ever known. An obvious tribute to his ability and maturity was the desire of the student body to have him, a man practically new to the school, as advisor to student council.

The untimely death of Dr. Schwind in his 46th year, even before some of us had had the opportunity to know him well, comes as a great blow and deprives the medical school of one of its best teachers in an area where inspiration has perhaps its highest value. The faculty feels keenly the loss of a respected and admired member.

G. L. STREETER. Dr. Streeter's biography is to be published separately in the *Anatomical Record*.

HAROLD LORRAINE WEATHERFORD, born 1891, died 1948. Assistant Professor of Anatomy, Harvard Medical School. Elected to membership in 1930. Dr. E. A. Boyden has submitted the following biography.

Harold L. Weatherford died suddenly on July 7, 1948, of a heart condition of which he had been aware and which he had treated with the equanimity of one who has learned to put

first things first. Indeed, not long after graduating from Stanford, in 1916, he had chosen to interrupt his career to support the family that had been deprived of a physician's income by the illness and premature death of his father. Thus coming late to the study of anatomy (lactation), in his 33rd year, under V. E. Emmel of Chicago, he proceeded to put into practice the principle that guided his whole professional career — to do thoroughly and unhurriedly that to which he set his hand. Under F. T. Lewis at the Harvard Medical School his interests in cytology broadened to include other anatomical disciplines including the history of anatomy. Soon the need for a greater knowledge of the technical methods of European histologists led him to Freiburg where he studied under v. Mollendorf, then perhaps the leading histologist in Germany. In Tübingen, he found Heidenhain and the "finest histological preparations I have seen." After returning to Boston he published in v. Mollendorf's *Zeitschrift* a meticulous treatment of the Golgi apparatus of the liver. About this time he became an Associate Editor of *The Anatomical Record*, in which capacity he served with great distinction for 16 years. In view of his critical judgment and command of the literature, it was no surprise when J. L. Bremer selected Weatherford to edit the new edition of the *Text Book of Histology* — the work by which he is presumably best known to anatomists outside his special cytological field. As a teacher of medical students he filled a unique position. One of them has said, "He was one of the Harvard instructors who was most remembered — We recognized in him a profound scholar who was greater than his academic position. He spent a long time talking with small groups of us, entertaining us with his subtle humor and stimulating us with his broad grasp of the significance of what we had hitherto considered mere details. Most of all, he liked us and lived his part." Thus he fulfilled in 57 short years the promise which his modest, generous nature would have claimed for others but never for himself.

FRANZ WEIDENREICH, born 1873, died 1948. Honorary Director, Cenozoic Laboratory, Peiping Union Medical College, Peking, China; Research Associate, American Museum of Natural History, New York. Elected to membership in 1940. Dr. Ernst Scharrer has submitted the following biography.

In few departments of human thought are myths and nebulous conjectures so meekly contested as they are in the field concerned with man's origin and early history. One of the reasons is that there are only few investigators who are in a position to contribute significant factual material and to interpret it with authority. The late Dr. Franz Weidenreich was one of them. His passing shortly after his 75th birthday, therefore, not only is mourned by his family, friends and colleagues, but it means the loss of an intellectual force that was felt well beyond the confines of morphology.

Weidenreich's monumental achievement came in the last decade of his life. His scientific biography, prior to his appointment as Director of the Cenozoic Research Laboratory of the Peiping Union Medical College, looks, in retrospect, like a well planned 40 years' course of study in preparation for the greater final thesis with which Weidenreich graduated to his permanent place in the history of science.

His earliest work in Strasbourg (1899-1901) was concerned with comparative neuroanatomy, specifically the cerebellar nuclei, the histology of the human skin, and the vascular system of the spleen. After a short interlude of work on cancer with Paul Ehrlich at Frankfurt, Weidenreich returned to Strasbourg for another 17 years during which he did most of his well known work on hemolymph nodes, leucocytes and blood platelets. He came for the first time to the United States in 1909 when he accepted an invitation from Minot to report on his hematological investigations at the AAAS meeting at Boston.

His interest in physical anthropology dates back to 1904 when he published a paper on the human chin. This interest became preponderant in the period between 1919, when Weidenreich was evicted from Strasbourg "because he was

a German," and 1933, when he had to migrate again "because he was not a German." During this time he worked, at Heidelberg and later at Frankfurt, on race and constitution, bone, teeth, phylogeny, lymphatics, etc. His publications are many and extensive and they are concerned with many fields of anatomy; he was indeed "rich in pastures," as his name implies.

After he left Germany and after a short stay at Chicago, Weidenreich went to Peiping, and it was there and at Java (with von Koenigswald) that he collected the material for his epochal on the history of the hominids. The war in the Pacific interrupted his studies once more, but did not prevent him from fulfilling the task he had set for himself.

Weidenreich has taught us a great deal about *Sinanthropus*, about blood, about the human foot and about many other subjects. But beyond that, by his very example, he gave us a profound lesson in what a scientist should be. We shall always remember him as a true one.

Members resigned: Ralph J. Bailey, Vincent J. Dardin, Allan F. Gutmacher, Alice E. Parker, Hans Selye.

The resignations were accepted with regret.

Members dropped for non-payment of dues for two years or more: The Secretary announced that the following two members had been dropped for non-payment of dues by action of the Executive Committee. Both of these members have been in arrears for 4 years. The Secretary has been unable to contact these members since the addresses in the files are incorrect and no one has been able to furnish information as to their whereabouts.

Clyde Marshall, Halifax, Nova Scotia, Canada
Jacob Thorkelson, Butte, Montana

(The Constitution, in Article VI, Section I, provides that former members "may be reinstated at the discretion of the Executive Committee on payment of arrears.")

Election of New Members: The Secretary presented the following 37 names of persons elected by the Executive Committee to membership in the Association, the names in parentheses indicating the sponsors.

- A. A. ALBERT, B.A., St. Stevens College, 1932; M.A., Harvard, 1933; Ph.D., Harvard, 1935; M.D., Harvard, 1943. Consultant in the Mayo Clinic. Director of the Hormone Assay Laboratory, Mayo Clinic. (George M. Higgins, W. Henry Hollinshead.)
- C. WILLET ASLING, A.B., 1934; M.A., 1937; M.D., 1939, University of Kansas; Ph.D., 1947, University of California. Assistant Professor of Anatomy, University of California. (Herbert M. Evans, Miriam E. Simpson.)
- MURRAY LLEWELLYN BARR, B.A., 1930; M.D., 1933; M.Sc., 1938, University of Western Ontario. Associate Professor of Anatomy, University of Western Ontario. (Eric Linell, F. L. McNaughton.)
- M. NOBLE BATES, A.B., Hamilton College 1932; A.M., Oberlin College 1936; Ph.D., Cornell University, 1943. Associate in Histology and Embryology, Daniel Baugh Institute of Anatomy, The Jefferson Medical College of Philadelphia. (George A. Bennett, J. Carsons Schaeffer.)
- VICTOR V. BRUNST, Doctor of Biological Science, University of Kiev, 1938. Research Associate, Department of Anatomy, University of Maryland Medical School. (Frank H. J. Figge, Eduard Uhlenhuth.)
- JESSE E. EDWARDS, B.S., 1932; M.D., 1935, Tufts College. Assistant Professor, Pathologic Anatomy, Mayo Foundation, University of Minnesota. (Benjamin Spector, Edward A. Joy.)
- THOMAS LLEWELLYN EVANS, A.B., 1925; A.M., 1931; Ph.D., 1936, University of Denver, Harvard University. Assistant Professor of Anatomy, Long Island College of Medicine. (Charles R. Noback, George H. Paff.)
- ELIZABETH FEKETE, B.A., Teachers College, Hungary, 1914; M.A., University of Michigan, 1929. Research Associate, Roscoe B. Jackson Memorial Laboratory. (Clarence C. Little, Paul B. Sawin, Katharine P. Hummel.)
- ROBERT A. GOOD, Ph.D., M.D. A three year fellowship in Pediatrics, the Whitney fellowship, University of Minnesota. (B. Campbell, A. T. Rasmussen.)
- EARL LE ROY GREEN, B.S., Allegheny College, 1935; Sc.M., Brown University, 1937; Ph.D., Brown University, 1940. Associate Professor of Zoology, Ohio State University. (Paul B. Sawin, C. C. Macklin.)
- WILLIAM EDWARD HAHN, D.D.S., University of Maryland, 1931, A.B., 1938; M.S., 1939, University of Rochester. Professor of Anatomy, University of Maryland, School of Dentistry. (C. L. Davis, George W. Corner.)
- MORGAN HARRIS, A.B., University of California, 1938; Ph.D., University of California, 1941. Assistant Professor of Zoology, University of California. (Herbert M. Evans, Miriam E. Simpson.)
- JAN JANSEN, M.D., Oslo University, 1924; Dr. Med., 1931. Professor of Anatomy, Oslo University. (C. Judson Herrick, Normand L. Hoerr.)

- ALEXANDER G. KARCZMAR, M.S., Warsaw University, 1939; M. A., Columbia University, 1941; Ph.D., Columbia University, 1946. Associate Professor, Georgetown University School of Medicine. (Oscar E. Schotte, Elmer G. Butler.)
- GRACE F. KATSH, B.A., N.Y.U., 1940; M.S., N.Y.U., 1944; Ph.D., N.Y.U., 1948. Instructor, New York University, Department of Biology. (Harry A. Charipper, Albert S. Gordon.)
- HAROLD KOENIG, B.S. cum laude, Rutgers; B.M. and M.D., Chicago Medical School; M. S. Northwestern University Medical School; Ph.D. University of Pennsylvania Medical School. Associate Professor of Anatomy, Chicago Medical School, 710 S. Wolcott St., Chicago, Ill. (William F. Windle, Roy G. Williams.)
- MAURICE LEV, B.S., New York University, 1930; M.D. Creighton University Medical School, 1934. Assistant Professor of Pathology, Creighton University School of Medicine, on leave of absence. (Cleveland C. Simkins, Leo P. Clements.)
- IAN WHITELAW MONIE, M.B., Ch.B., University of Glasgow, Scotland, 1940. Assistant Professor of Anatomy, University of Manitoba, Department of Anatomy. (Maclaren Thompson, Donald J. Bowie.)
- JEVZY OLSZEWSKI, Physician's Diploma, Stefan Batory University, Wilno, Poland, 1937; M.D. University of Freiburg, Berlin, Germany, 1946. Research Fellow, Montreal Neurological Institute. (Francis L. McNaughton, Roy L. Swank.)
- EDMUND CONVERSE PEIRCE, 2ND, S.B., Harvard College, 1940; M.D., Harvard Medical School, 1943. Assistant Professor of Anatomy, The Johns Hopkins University School of Medicine. (Allan L. Grafflin, William L. Straus, Jr.)
- ROBERT DURANT RAY, A.B., 1936; M. A., 1938, University of California; M.D., 1943, Harvard University; Ph.D., University of California, 1948. Assistant Professor of Orthopedic Surgery (on leave of absence); at present Assistant Resident in the Department of Orthopedics, University of California Hospital. (Herbert M. Evans, Miriam E. Simpson.)
- HEINZ ROLLHÄUSER, M.D., University of Freiburg/Br., Germany, 1944. Prosector, Department of Anatomy, University of Marburg/Lahn, Germany. (Warren H. Lewis, E. J. Farris.)
- JOHANNA TER HORST ROLLHÄUSER, Ph.D., University of Freiburg/Br., Germany, 1945. Assistant, Department of Anatomy, University of Marburg/Lahn, Germany. (Warren H. Lewis, E. J. Farris.)
- LEWIS P. ROWLAND, B.S., Yale University, 1945; M.D. Yale University School of Medicine, 1948. Assistant in Medicine, Yale University School of Medicine. (W. U. Gardner, Fred A. Mettler.)
- MEREDITH N. RUNNER, Ph.D., Indiana University, 1942; Research Associate, Roscoe B. Jackson Memorial Laboratory. (Clarence C. Little, Katharine P. Hummel.)
- AMELIA SAMANO (BISHOP), Dr., Teaches Human Embryology in the Medical School and Histology in the Graduate School, National University of Mexico. (F. Gaynor Evans, Ernest Gardner.)
- JØRGEN ULRIK SCHLEGEL, M.D., 1945; Ph.D., 1948, University of Copenhagen. Associate Professor in histophysiology at the University of Copenhagen, Medical School. (George W. Corner, Samuel R. Reynolds.)

- ROBERT OLIVER SCOW, A.B., 1943; M.A., 1944; M.D., 1946, University of California. Senior Assistant Surgeon, U.S. Public Health Service at National Institute of Health. (Herbert M. Evans, Miriam E. Simpson.)
- BENJAMIN G. P. SHAFIROFF, M.D., Long Island College Hospital, 1929. Associate Professor of Anatomy, New York University, Bellevue Medical Center. (Donal Sheehan, Pinckney Jones Harman.)
- FERN WENONAH SMITH, A.B., Smith College, 1940; M.S., Brown University, 1942. Ph.D., University of Minnesota, 1945. Assistant Professor of Anatomy in charge of Embryology and Histology, University of Missouri. (M. D. Overholser, W. U. Gardner.)
- R. DALE SMITH, B.S., University of Pittsburgh, 1936; M.S., University of Pittsburgh, 1938; Ph.D. University of Pittsburgh, 1939. Assistant Professor of Gross Anatomy, University of Maryland Medical School. (Eduard Uhlenhuth, Frank H. J. Figge.)
- PHILIP THOREK, B.S., 1928; M.D., 1930, University of Illinois; F.A.C.S. 1938; Diplomate, American Board of Surgery, 1940; F.I.C.S., 1946. Assistant Clinical Professor, Department of Surgery, University of Illinois, Associate Professor of Surgery, Cook County Graduate School of Medicine, Associate Attending Surgeon, Cook County Hospital, Senior Attending Surgeon, American Hospital and Alexian Bros. Hospital; Assistant in Gross, Microscopic and Topographic Anatomy, University of Illinois College of Medicine, 1933-1941; Assistant Professor of Surgical Anatomy, Cook County Graduate School of Medicine, 1933 to present. (Otto Kampmeier, Arnold Albert Zimmermann.)
- JOHN J. TRENTIN, B.S., Pennsylvania State College, 1940; A.M., 1941, Ph.D., 1947, University of Missouri. Jane Coffin Childs Fellow, Anatomy Department, Yale Medical School. (W. U. Gardner, L. S. Stone.)
- CHARLES VAN BUSKIRK, A.B., Westminster College, 1939; M.S., St. Louis University, 1941; Ph.D., University of Minnesota, 1943; M.D., Albany Medical College, 1947. At present Resident in Neurology. Was Instructor of Anatomy (Albany) 1944-1947, Teaching Assistant in Anatomy, University of Minnesota, 1941-1943. (A. T. Rasmussen, E. A. Boyden.)
- ELIZABETH LLOYD WHITE, A.B., Goucher, 1937; M.A., Bryn Mawr, 1938; Ph.D., 1947. Instructor, Washington University, St. Louis. (Jane M. Oppenheimer, Viktor Hamburger.)
- GEORGE M. WYBURN, D.Sc., Glasgow, 1938; M.B., Ch.B., Glasgow, 1925. F.R.F.P.S.G., Glasgow, 1928; F.R.S.E. Regius Professor of Anatomy University of Glasgow. (W. J. Hamilton, J. D. Boyd.)
- ANITA ZORZOLI, A.B., Hunter College, 1938; M.A., Columbia University, 1940; Ph.D., New York University, 1945. Assistant Professor, Washington University School of Dentistry, St. Louis. (Roberts Rugh, Arnold Lazarow.)

On motion, duly seconded and passed unanimously the election of these new members was ratified.

Members placed in the category of "members relieved of the payment of dues": The Secretary reported that, by action of the Executive Committee, 9 members had been placed in this category in accordance with the provisions of Article VI, Section II, of the Constitution, which reads: "Any member who has paid the annual dues for 30 years, or who has attained the age of 65 years, or who has retired because of illness, may be relieved of the annual dues at the discretion of the Executive Committee." It was stated that this brings to 44 the total number of persons in this category.

Number of members:

Total membership April 21, 1948	885
Deceased during year	9
Resigned during year	5
Dropped for non-payment of dues	2
	16
	869
New members elected April 14, 1949	37
	906
Total membership April 14, 1949	

Payment to Local Committee: The Secretary presented the recommendation of the Executive Committee that the Local Committee be allowed \$600.00 to defray costs of the meeting. On motion duly made and seconded it was voted that the Treasurer be authorized to pay the sum of \$600.00 from the general funds of the Association to the Local Committee.

Place of next meeting: It was announced on behalf of the Executive Committee that the Committee had voted to accept an invitation from Louisiana State University, New Orleans, to meet there in 1950, April 5, 6, and 7. The headquarters hotel will be the Jung.

Report of Delegate to American Association for the Advancement of Science: Dr. F. H. J. Figge attended the A.A.A.S. meeting in Washington, D.C., September 13, 1948, as a delegate of the American Association of Anatomists. The following is excerpted from Dr. Figge's report:

"The council of the American Association for the Advancement of Science met Monday, September 13, 1948, in Washington, D.C. The president of the Association, Dr. Edmund W. Sinnott, announced appointment of Dr. Howard A. Meyerhoff as administrative secretary to succeed Doctor F. R. Moulton who retired after having served in this capacity for many years. Doctor Moulton reviewed some of the facts concerning the growth of the organization. He pointed out that it had grown from a membership of 461 in 1848 to 41,705 in 1948. The Association receives and spends approximately \$500,000 per year.

It was voted to authorize the general secretary to appoint a committee to study and recommend standards or qualifications upon which societies may be considered for affiliation with the Association.

Dr. Baitsell raised the question of the advisability of continuing the method of starring scientists in American Men of Science. Dr. Carlson also raised the same question concerning the custom of electing members to fellowship in the Association. It was voted to poll the membership of the Association to determine whether they favored the continuation or abolition of these customs or whether they favored modifications involving the adoption of minimum uniform standards for election to fellowship and the establishment of uniform criteria for starring scientists.

The other items discussed were the election of a president, the type meeting preferred, the establishment of local chapters of the A.A.A.S., the possibility of an international federation of associations for advancement of science and the Gordon Research Conferences at New London, New Hampshire.

Most of these matters were referred to committees for reports or study."

Dr. Davenport's report as representative of the Association to the Stain Commission was not read at the meeting but is presented herewith:

"The last regular meeting of the Trustees of the Stain Commission was held at the Medical School of the University

of Rochester, February 28, 1948. The next meeting is scheduled for April 16, 1949, in the Department of Anatomy of the University of Pennsylvania.

Dr. H. J. Conn, the President, retired from his position at the Agricultural Experiment Station, Geneva, New York, May 1, 1948, and has been giving full time to the Stain Commission. All physical connection with the Station has been discontinued, since Dr. Stotz, the Treasurer, has gone to the Biochemistry Department of the University of Rochester as Department Head, and Miss Darrow, who had for many years been doing the biological testing of stains, was given laboratory facilities there also. Offices for the Stain Commission were established in a Geneva office building in October 1947. The Commission's publishing agency, *Biotech Publications*, is located in these offices.

The journal *Stain Technology* has grown from an annual volume of 150-160 pages to 232 pages for 1948. The additional costs of publication have been defrayed by a one dollar a year increase in subscription rate, and by the sale of more advertising space. A publication committee consisting of Louis Gershenfeld, Chairman; H. A. Davenport and E. H. Stotz was appointed by Dr. Conn last April.

The question of research grants was discussed at the last meeting, and it was agreed that the Commission had sufficient funds to support more research, should suitable projects and requests for aid come to its attention."

Appropriation of \$100 to the National Society for Medical Research: It was again voted to pay the National Society for Medical Research \$100 in recognition of the splendid work of this society.

Report of the Committee on Anatomical Nomenclature: Dr. Corner read the following report:

"After meeting annually for three years and carefully examining the question of a revision of the nomenclature of gross human anatomy, the Committee remains divided as to the type of revision which is desirable and as to the wisdom of preparing a revision at the present time. The general

membership was also divided in the ballot taken by the Committee at the meeting of 1948, precisely one-half of those present voting for a nomenclature using the comparative-embryological orientation, and half for a moderate revision of the BNA. Since the question of revising the nomenclature was revived in our association, there has been an improvement in the international situation which suggests a cautious policy. The International Congress planned for 1950 may raise the question of revising the BNA. If so, it would be better for us not to be committed to any specific program. The Committee reports therefore that it proposes to take no further steps at present."

GEORGE W. CORNER
Chairman

MINUTES OF THE SECOND BUSINESS SESSION

April 15, 1949, 12:30 P.M.

Motion Picture Committee: Dr. J. E. Markee made the following statement:

During recent years a considerable number of motion pictures and film strips have been produced which are designed for the teaching of anatomy. Most of these teaching aids have not been submitted to the Motion Picture Committee for listing. The existing Motion Picture Committee has concerned itself primarily with research films.

It is advisable that a second Motion Picture Committee be founded to collect and disseminate information about visual aids to those who produce and those who use motion pictures in the teaching of anatomy. The task is a double one. First, information should be collected and disseminated regarding the existing films that are not currently listed because of the expense to the producer. A number of these unlisted films can be purchased from the maker of the films. The information regarding all of these anatomical films should be made available to all of the anatomists. Second, information about existing films should be made available to those who are producing new films. Already there is some duplication. Further

duplication should be of the planned type so that additional films on any anatomical topic will be different from and better than the existing films.

A number of the members of the Association have expressed their willingness to undertake this task as individuals if the Association does not care to authorize the formation of a committee on audiovisual aids in anatomy.

No action was taken on this motion. However, in response to the suggestion made at the Friday Business Session, April 15, 1949, the Motion Picture Committee, consisting of W. H. Lewis, J. E. Markee, C. C. Speidel and E. R. Clark (Chairman), presents the following statement:

In 1940, the Wistar Institute of Anatomy and Biology, under Dr. Edmond J. Farris, created at the Wistar Institute a depository for motion picture films of anatomical and zoological interest. The main purpose of the original plan was to provide a central location where films would be available to research workers. However, Dr. Farris had in mind the possibility that the plan might also become useful to anatomists and zoologists as a source of information regarding films available for both teaching and research. The American Association of Anatomists was invited to select a Committee which would assist him by reviewing all anatomical films submitted. A Committee of three was elected in 1940, which has been reelected annually to date, with the addition of a 4th member at the 1949 meeting.

The scheme has provided a central depository and clearing-house for biological motion picture films. It has been of greatest use in connection with teaching, because films made originally as research records often become extremely useful for teaching.

There are many valuable motion picture films, of both research and teaching interest, that have not yet been submitted for review. This Committee suggests that producers, who possess such reels and would like to make them available to others, send them to Dr. E. J. Farris, The Wistar Institute of Anatomy and Biology, Woodland Avenue and 36th Street,

Philadelphia 4, Pennsylvania, for consideration and, if approved, for listing, with other approved motion picture films, in *The Anatomical Record*. For style of listing consult *The Anatomical Record*, Vol. 101, May 1948. For details write to Dr. Farris.

(Signed) ELIOT R. CLARK
for the Committee

The American Institute of Biological Sciences: As our representative to the governing board of the A.I.B.S., Dr. Louis Flexner read the following report on the activities of this society for the past year:

“The American Institute of Biological Sciences:

1. Arranged a convention of 11 biological societies in Washington, D. C. The anatomists were not represented at the convention.

2. Collected funds for a study of the publication needs of biological journals. Anatomists will be interested in this at the present time to the degree that they publish in and read biological journals other than the anatomical journals.

3. Placed its Committee on Selective Service on General Hershey's panel. The recommendations of the A.I.B.S. committee, which provide for deferment of a large proportion of the better students of the sciences and humanities at all institutions, has been adopted by General Hershey's panel and is now being reviewed at higher levels.

4. Initiated several special projects of which the Handbook of Biological Data, supported by funds from the Air Material Command, is the most pertinent to anatomists.”

Through the kindness of Dr. Zwemer the following report was circulated among the members.

Purposes: The purposes of the Institute are the advancement of biological sciences and their applications to human welfare. To serve these purposes, the Institute will, among other things, assist societies and other organizations in such matters of common concern as can be dealt with most effectively by united action; co-operate with local, national and international organizations concerned with the biological sciences; promote unity and effectiveness of effort among all those who are devoting themselves to the biological sciences and their applications by research, by teaching, or by study; and, foster the relations of the biological sciences to other sciences, to the arts and industries, and to the public good.

ACTIVITIES OF THE INSTITUTE 1948-1949

Handbook of Biological Data: The first section on blood composition and reaction is scheduled to be compiled within the next 8 months. Other sections will be initiated as quickly as possible. The Handbook will contain authentic, accepted, and frequently used constants, data, normal values, tolerances, and standards applying to the quantitative aspects of biology in its broad and basic sense, including many fields of applied biology.

Inter-Society Color Council: The AIBS has been asked to represent the field of biology. The purpose and aim of the Council is to stimulate and coordinate the work being done by the various societies, organizations, and associations leading to the standardization, description, and specification of colors and to promote the practical application of these results to the color problems arising in science, art, and industry. A questionnaire will be sent soon to the Head of Departments of the Biological Sciences of Universities, Research Institutes, Museums, and Arboreta to determine the interest of biological scientists in color standardization.

Utilization of Scientifically Trained Personnel by the Military: The AIBS Committee on Selective Service was asked by the Director of Selective Service to serve as his advisory panel in the field of biology. This recognition and utilization marks the first time that biology as such has been recognized in connection with manpower problems in the United States. The report "Scientists in Uniform, World War II," a cooperative undertaking by the Department of the Army and 31 scientific societies, directed by Dr. David M. DeLo has resulted in the Logistics Division, General Staff, Department of the Army requesting the AIBS to form a committee to advise on the proper utilization of biologists by the Military.

American Type Culture Collection: The Institute in cooperation with the Division of Biology and Agriculture, National Research Council, set up a committee to study the ATCC and recommend measures to place the ATCC on a sound financial basis and to secure funds.

Joint Meetings: In September, 1948, the AIBS sponsored a joint meeting of 11 biological societies. As a result of this meeting the Institute has been requested to sponsor a joint meeting in 1950. The meeting will not be limited to member societies and any biological society may participate. A definite decision as to time and place will be made shortly.

Cooperative Printing: At the request of several of the biological societies, the AIBS is investigating the possibilities of a joint cooperative printing enterprise. Journals will be standardized as much as possible in physical characteristics, but the Societies will retain editorial control of the journal. Printers have been contacted and several have shown interest. It is hoped to start the effort by January, 1950. The Committee on Publication Problems is also studying the possibility of establishing a joint central editorial and business office to relieve the Editors (after manuscripts have been accepted), Secretaries, Treasurers, and Business Manager of the Societies of much routine work.

Information of Importance to Biologists: Any information regarding legislation such as postal rates, national science foundation, Swan Island Quarantine Station, the relations of biology to other sciences, etc., are sent to the Societies

immediately upon receipt, and a notice of the information printed in the AIBS News Letter. The News Letter at present is sent to the Officers of the Societies and the Council or Executive Committee. Eventually it is hoped to send the News Letter to every member.

Addressograph System: The Institute proposes to initiate in the next few months an addressograph plate system of all members. The plates will be available for Society use.

Total Membership: As of January 1, 1949, the Institute had 15 member societies and two affiliate societies. The total number of members is approximately 11,000 unduplicated and if duplicate membership is counted, the Institute has approximately 18,000 members.

It was voted to renew our membership in the AIBS for another year.

Dues for 1949: The Secretary-Treasurer reported that the Executive Committee had recommended that the dues for the year 1949 be raised to \$3.50. This is \$.50 above the \$3.00 level which has existed in 1948, and was suggested in order to provide for membership for one year in the American Institute of Biological Sciences.

It was voted to renew our membership in the AIBS for another year.

Dr. Wislocki presented the following resolution, which was adopted unanimously:

"Dr. Bartelmez and Members of the Society:

On behalf of the members and officers of the American Association of Anatomists, I have the pleasant duty of thanking the Local Committee from Temple University School of Medicine for their hospitality and the splendid organization of the meeting. The Committee's efforts have contributed materially to making our visit to Philadelphia a happy one, and we extend a vote of thanks to Dr. Huber and his associates. We wish also to express our appreciation for their hospitality to Lea and Febiger, the Blakiston, J. B. Lippincott and W. B. Saunders Companies, as well as to the Philadelphia Coca-Cola Bottling Company."

Report of the Committee on the Economic Status of Anatomists: Dr. Gordon Scott read a summary of the report of this committee. The report in full is printed herewith. Then Dr. Scott made the following motion:

"Your committee concludes from its study that the present status of Anatomy is sufficiently alarming that efforts must be made to improve the situation. We are convinced that our science does not stand alone in its present condition and that its unenviable position is shared by the other preclinical sciences — physiology, pharmacology, biochemistry, bacteriology and pathology. With these beliefs in mind, we propose:

1. That your committee be empowered to contact a foundation or foundations, or other agencies and ask support for a survey of the status of the preclinical sciences so that definitive proposals can be made for their improvement.

2. That if such support can be obtained, the foundation or agency work with a committee of representatives appointed by the various societies in the preclinical sciences including the American Association of Anatomists."

After being duly seconded, this motion was carried.

THE MEETING OF THE EXECUTIVE COMMITTEE

April 12, 1949

Business transacted by the Executive Committee, but not transmitted to the membership at the two business sessions, because of lack of time, included the following items:

Report of the committee appointed to study the length of the presidential term:

"It is the considered judgment of the committee that the major function of the American Association of Anatomists is to promote the advancement of science in general and of anatomy in particular. To hold an annual meeting for the presentation and discussion of studies in science and education and for the election of officers is an important part of this function. To the best of our knowledge the present system of electing a president for two years and a secretary-treasurer for 4 years is functioning effectively in meeting our objectives. We recommend that this practice be continued."

W. E. SULLIVAN, *Chairman*

J. S. NICHOLAS

NORMAND L. HOERR

Anatomical Journal Trust Fund: (See financial report.) The committee of three to decide expenditures from this fund is Patten, Smith and Mason. Dr. Mason was to serve for one year only but was re-elected for a term of three years.

National Research Council Representative: Dr. Bartelmez's nomination of Dr. George W. Corner as representative of the American Association of Anatomists in the division of Medical Sciences of the National Research Council for 1949-1952 was approved.

It was suggested that the membership be notified of the proposed International Anatomical Congress to be held at Oxford, England, July 25-28, 1950. The following letter has been received:

"The Committee are most grateful to all those who replied to the questionnaire asking for an expression of views regarding the holding of an International Anatomical Congress.

I am now writing to say that it has been decided to hold such a Congress in Oxford from Tuesday, 25th July, to Friday, 28th July, 1950. These dates have been selected in order that the attendance at the XVIII International Physiological Congress, which is being held at Copenhagen from 15th to 18th August, may not be adversely affected. It is also understood that the Steamship "Queen Elizabeth" is scheduled to arrive at Southampton on 20th July.

The programme has not yet been arranged, and will depend largely on the numbers of papers to be presented in the different Sections. It is intended to include a Symposium on Nomenclature in relation to Human Anatomy, and it is probable that Sections will include the following subjects: General Anatomy, Histology and Embryology, Morphogenics and Endocrinology, Neurology and Physical Anthropology.

In addition, provision will be made for the demonstration of apparatus, etc., and for the projection of cinematographic films.

Membership of the Congress will not be confined to members of Anatomical Societies, but will be open to persons

working in approved institutions who are engaged in bona fide research or teaching of any of the subjects included in the programme. The Membership Fee will be £ 2. 10. 0.

Accommodation for the majority of the members will be provided in Colleges at a cost of approximately £ 1. 5. 0. per day, and will be arranged by the Congress Office. It will, therefore, be essential that those who intend to be present should intimate the fact not later than the date which will be indicated for this purpose, in order that the correct number of rooms may be reserved in advance. Details on this and other points will be furnished at a later date when the actual invitations are issued."

(Signed) GRAHAM WEDDELL

Congress Secretary

Arrangement of Programs: The Executive Committee voted that the Secretary be empowered to select a committee for the arrangement of the program of the annual meeting. In view of the fact that recent programs have grown beyond the capacities of medical schools and hotels, this committee is authorized to limit the number of papers and demonstrations to be presented.

NORMAND L. HOERR

Secretary

REPORT OF THE COMMITTEE ON SURVEY OF ECONOMIC
STATUS AND PROFESSIONAL OPPORTUNITIES
OF ANATOMISTS

Recruitment of medical scientists is one of the foremost obligations of medical educators. It is not enough that our energies be devoted to the education of physicians. We are all repeatedly assailed by demands for more doctors, for less emphasis upon research and more upon what the shortsighted claim to be our primary task. We all know that good teaching and scholarly research are inseparable, and each is a function of every department within the University. It is to be hoped that we all still believe that progress in medicine is determined by the achievements of research in the basic sciences. If this fundamental premise be sound, then the *recruitment* into and the *retention* of first-rate minds in the basic sciences—and one most certainly includes anatomy among them—becomes a matter of utmost and immediate importance. We realize that if none but the mediocre work in these areas, advancement in medical science will not only be retarded, but will cease. It is a truism which must be constantly reiterated that medicine and basic science are concomitant in their progression and that the fundamental sciences set the pace.

Your committee began with the assumption that the foregoing statements were true, and, therefore, acceptable to the members of the association. We believe that the problems of retaining good young men in science is not one peculiar to the field of anatomy. We have also discovered that the problem is not unique to the period in which we find ourselves, but has caused concern to other generations. We are certain that at the present time its solution is inextricably associated with economic, social and political trends.

It is a matter of grave concern to all that learning still remains an occupation with little inherent social status in the United States. This serves to make a career in scientific medicine less attractive than it might otherwise be. Community approbation goes to the man of action and not to the fundamental scientist whose unceasing labor has made his success possible. When this is coupled with the general economic disadvantage of a scientific career, it becomes doubly difficult for a young man to elect a career in a subject like ours. A not inconsiderable weight in the balance against us is that most medical schools are located in large cities where university people form but a small fraction of the population and must live in social competition with those whose means are many times greater. It is, therefore, difficult to find adequate and deserved social recognition in a society whose measure of usefulness and success is an entirely foreign one. Many young men with inquiring minds and great ability feel that it is not quite right that their wives and children be asked to live on an academic salary when they could have the economic security that their larger earnings in business could afford. While many of them would be content to accept considerably less than they could otherwise earn, the existing differential is enough to discourage them.

There seems to be a firmly rooted conviction—by this time almost dogma—that university teachers are not entitled to financial returns commensurate with their contributions to society. This notion has developed to the point where it is now an accepted part of our folklore and has a literature and drama of its own. As far as the committee can determine there is no great difference in the birth rate in the families of scientists and other specialists. The dietary requirements of their children are, as far as available data indicate, the same as for others of our population. To put it briefly, there is every reason to believe that the fundamental scientist, too, is human with all the desires and needs and ambitions of his brother specialists. It would seem that the self-reproduction,

as well as the spontaneous birth of such contributors to human welfare, is a thing to be encouraged.

Your committee has not been insensitive to another important aspect of the whole problem of scientific recruitment in the medical sciences. If, as is not entirely unlikely, some form of voluntary or compulsory health insurance is established under government auspices, the demand for medical care will far exceed the capacity of existing medical facilities. Immediately the hue and cry to train more doctors will become more insistent. It has already reached considerable proportions. It will be difficult, but not impossible, to find clinical teachers to meet the need; but where will be found the teachers in the basic science?

Your committee was convinced that, as a starting point, it would be well to study the present status of anatomy in medical schools and to learn how many men will be needed in the foreseeable future to maintain it. Accordingly there were selected from the 1948 list of members of the Association all those actively engaged in anatomy departments in medical schools in the United States. These are arranged by rank in figure 1.

These data are alarming for they indicate that no one of the lower ranks is large enough to replace the rank above it, and show, furthermore, that the shortage is most pronounced at the instructor level, where 20 individuals are now on hand to fill a total of 328 more advanced positions which will gradually become open. It should be indicated that a number of persons with the rank of instructor may not be members of the Association, and to this extent the data given deviate from the truth.

The temporal features of this opening of positions may be discussed after age, with which it is bound up, is considered.

Age in 1948, was determined from the American Men of Science, and is practically complete for the ranks of professor and associate professor. Of the assistant professors, a third were not listed and are assumed to be below the age

of 35. So few instructors were listed that they are not included.

The age data are charted in figure 2. These figures support the impression that age and rank go hand in hand. Thus, the largest number of assistant professors are in the decade ending at 35, of associate professors in the decade ending at 45,

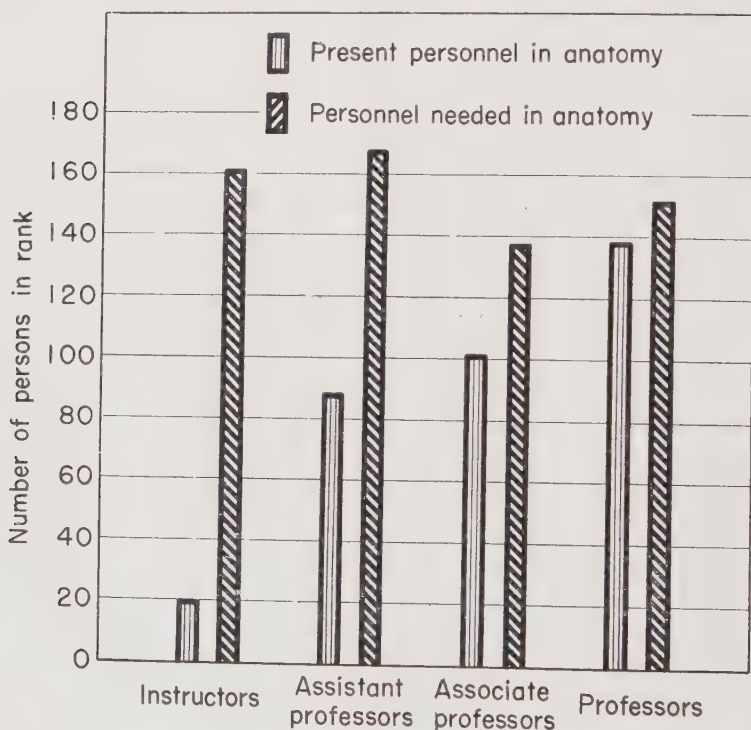


Figure 1

while the largest number of professors are in the decade ending at 55. As a matter of passing interest, this last item is indicative of the large number of young professors; 25 are between 36 and 45; 51 between 46 and 55; and 40 are in the decade between 56 and 65.

To consider the opening of positions, with the usual retirement age of 65, 40 professorial positions will come open

in the next 10 years. In addition there are 15 individuals over the age of 65 who presumably will retire in the next decade. Even if no unanticipated losses occurred, there will be a total of 55 professorial positions which will become open, from retirement, within the next 10 years.

If no more than a one-to-one ratio of replacements were advocated, at least 55 young men should be recruited into

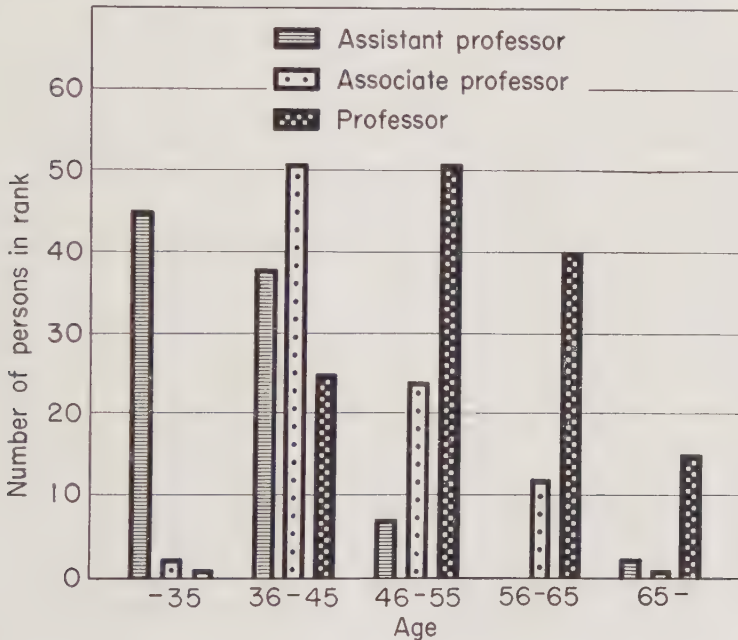


Figure 2

the profession during this 10 year period. In the past, the replacement ratio in anatomy has been higher than one to one, however, for a present total of 209 sub-professorial appointees compares with 139 professors—a ratio of exactly three to two.

If a replacement ratio of three to two be advocated, a total of 82, rather than 55, new men should be recruited into the profession in the next 10 years to do no more than maintain it at its present status. If it becomes necessary to expand the

profession, or if it seemed desirable to adopt a policy whereby an excess of young men be recruited and only the more capable of them advanced, this number should be correspondingly increased.

In order that your committee might gain enough information to assay existing and future needs, a questionnaire was sent to 76 department heads. The reason for this selection was that parts of the questionnaire dealt with salaries and budgetary matters and could be answered more accurately by departmental executive officers. Your committee realizes that far different answers to some of its questions might have been secured from anatomists in the younger group. It is confident that this group might well have given suggestions for improving the profession or attracting young men into it which were more to the point and showed less evidence of wishful thinking. The return of 57 questionnaires, or 70% was taken as an overall interest in the work of the committee, and a genuine desire to improve conditions, generally.

Existing vacancies in anatomy departments

The data secured will be dealt with in more or less generalized categories. It was shown that there are places now vacant for 107 persons with varying degrees of anatomical training. These are divided as follows: 5 professors, 8 associate professors, 36 assistant professors and 58 instructors. As might have been predicted from the data shown in figure 1, the greatest need is in the beginning ranks. This reveals that at the present time, anatomy is short by over 30% of filling its existing vacancies, for 107 such vacancies exist as compared with 328 present appointments. In terms of recruitment, 107 individuals are needed immediately in anatomy and 82 more will be needed in the next decade, making a total of 189 to do no more than fill existing vacancies and those coming open from retirement and in other ways. As will be seen later, a strikingly inadequate number of individuals is at present being trained to meet this deficiency.

Our next question may have been somewhat ambiguous and may well have resulted in duplication in figures regarding the needs in personnel. It was your committee's intent to discover also the number of personnel needed in addition to existing vacancies for optimum functioning of anatomy departments. In the discussion which is to follow immediately, we are assuming that the persons answering interpreted the question in the same manner as the committee. Anatomists felt that some 217 (62% more) added personnel would be needed to bring their staffs up to a level at which teaching would not be excessive, and at which adequate opportunity would be provided for research. This group was divided as follows: 8 professors, 27 associate professors, 51 assistant professors, 83 instructors and 51 technicians. It is of some interest to add these figures, together with those of existing vacancies, to those of the corresponding ranks shown in figure 1. The results are shown in the darker columns of the histogram in figure 1.

Thus it becomes an almost mathematical certainty, even with the added personnel thought to be needed, that *any* young person entering anatomy, regardless of qualifications, can expect to become an associate professor before the age of 45. This almost certainly follows if recruitment continues at its present rate and positions become vacant due to normal processes. It will be speeded up materially if the expected demand for training additional physicians becomes reality instead of wish. While it may seem to be a good thing to all young instructors to have assured futures, from the point of view of the development of ours and allied sciences, it is doubtful whether this would be so.

Present compensation for anatomists

Present minimal salaries for instructors range from \$1,500 to \$4,200 yearly, while maximal salaries range from \$2,300 to \$5,000. The averages are \$2,855 minimal and \$4,053 maximal with the modal figures being \$3,000 and \$4,000 respectively. Assistant professors fare somewhat better with

minimal salaries ranging from \$2,500 to \$5,400 and maximal salaries ranging from \$3,600 to \$6,200 with the modal salary being \$4,000 and \$5,000 respectively. In the ranks of associate professors the salary scales are not too bad in the maximal bracket. The minimal salary range is from \$4,000 to \$6,200 and the maximal range is from \$4,900 to \$10,000. It is safe to say that there are not many associate professorial salaries of \$10,000. The minimal salaries in this rank average \$4,889; and the maximal \$5,929. The modal numbers are \$5,000 and \$6,000 in the minimal and maximal brackets. Professors at present are paid salaries that range from \$4,000 to \$10,000 in the minimal bracket and from \$5,500 to \$15,000 maximal. The averages are \$5,533 minimal, and \$8,303 maximal. The modal salaries are \$6,000 minimal and \$10,000 maximal. Again, the highest salary figure of \$15,000 was not notable for its frequency of occurrence in the series. The modal salary of \$10,000 is not uncommon, as it seems to be a figure determined as one beyond which the earnings of the basic scientist — at least the anatomist — ought not, for the sake of common decency to go.

TABLE 1
Staff Salaries

		PRESENT		DESIRED	
		Minimum	Maximum	Minimum	Maximum
<i>Instructor</i>	Mean	\$2,855	\$4,053	\$3,423	\$4,521
	Median	2,800	4,000	3,500	4,500
	Mode	3,000	4,000	3,000	4,250
	Range	1,500-4,200	2,300-5,000	2,300-4,800	3,000-6,000
<i>Ass't Prof.</i>	Mean	3,995	4,950	4,602	5,986
	Median	4,000	5,000	4,500	6,000
	Mode	4,000	5,000	5,000	6,000
	Range	2,500-5,400	3,600-6,200	3,000-6,000	4,000-9,000
<i>Assoc. Prof.</i>	Mean	4,889	5,929	6,098	7,668
	Median	5,000	6,000	6,000	7,700
	Mode	5,000	6,000	6,000	7,700
	Range	4,000-6,200	4,900-10,000	5,000-8,000	6,000-10,000
<i>Professor</i>	Mean	6,633	8,303	7,749	10,810
	Median	6,500	8,000	7,500	10,000
	Mode	6,000	8,000	7,500	10,000
	Range	4,000-10,000	5,500-15,000	5,500-12,000	6,500-20,000

Salaries thought to be desirable. The committee was of the opinion that the answers to this part of the questionnaire were either given with becoming modesty or with the conviction that it would not do much good to raise a fuss about it anyway. Combined with these considerations there seemed to be some thought given as to what the traffic would bear. It was certainly not the intent of the committee that the data collected would be used as a nutcracker to put the squeeze on university administrative officers. Most of them are sympathetic with the problem, but there is nothing that can be done about it. Resources are limited in all universities. Their medical schools are expensive and many of them are coming to believe that they neither are able, nor should be expected to carry the burden of medical education. It would seem that the problem is a national one, and its solution may well lie in a complete appreciation of the values of education in all of its branches. The desired increases for instructors ranged from \$100.00 to \$1,500 in the minimal, and \$200.00 to \$2,000 in the maximal brackets. The averages were \$687.00 and \$942.00 respectively. or assistant professors, average increases in minimal and maximal scales of \$852.00 and \$1,072 were thought needed. These ranged from \$200.00 to \$3,000. Associate professors, according to the replies should have salary increases of from \$500.00 to \$3,000. The needs of professors were evaluated anywhere between \$100.00 and \$7,500.

Research funds. Answers to this question revealed a most surprising and shocking situation; for more than two-thirds of the anatomy departments in the country the University budget appropriation for research totalled only \$96,500. Thirty percent of these departments have no research appropriation, and 10% were unable to separate research appropriation from the remainder of the budget. One suspects that research in these latter departments has either ceased, or is carried on in clandestine cells. This, in reality, means that in only 34 of the medical schools represented in our data is research considered to be vital to anatomy and therefore

to medical science. Poverty does not excuse this lack of sympathy with the objective of our science. This leaves ignorance as the only plea. Those medical schools who do actively support research in anatomy as a normal part of their obligation, do so in annual amounts which range from \$300.00 to \$8,200. The committee doubts that this poor showing can be matched by any of the other fundamental sciences.

TABLE 2
Question 1 *Increases in salary desired*

		ABOVE PRESENT MINIMUM	ABOVE PRESENT MAXIMUM
<i>Instructor</i>	Mean	\$687	\$942
	Median	600	1,000
	Mode	500	1,000
	Range	100-500	200-2,000
<i>Ass't Prof.</i>	Mean	852	1,072
	Median	600	1,000
	Mode	500	1,000
	Range	200-2,000	200-3,000
<i>Assoc. Prof.</i>	Mean	1,055	1,395
	Median	1,000	1,000
	Mode	1,000	1,000
	Range	500-2,500	300-3,000
<i>Professor</i>	Mean	1,500	2,400
	Median	1,100	2,000
	Mode	1,000	2,000
	Range	100-4,000	750-7,500

Twenty-five percent of the departments included in this survey received no grant-in-aid monies from sources outside the school. The remaining 75% received a total of \$582,680. Ten departments spend more than \$15,000 annually from grants-in-aid. The range of grant-in-aid money for departments varied from \$1,000 to \$87,190. Thus the total research monies expended by the 57 anatomy departments furnishing data was only \$679,180, of which 14% came from departmental appropriations and the remaining 86% came from outside sources. By way of comparison, one might easily name a

half-dozen departments of biochemistry whose research spending would total that of all the 57 anatomy departments under consideration combined. Had the other 30% of anatomy departments cooperated with your committee, it is a practical certainty that we would not have been able to muster over a million dollars as total for annual research expenditure in our subject in this country. Your committee, by way of conjecture, would like to add that in all probability one-half or more of this available research money is spent in the fields of cancer and endocrine investigation.

Some measure of the outlook in anatomy can be had from the fact that 15% of the 57 departments desired no increase in research budget and in 10% the answers were not clear enough for the committee to make much sense from them. The remaining 75% of the departments wished increases in amounts varying from 10 to 2,000%. Some departments having no research budgets wanted none; some having none felt that with present facilities they could expand but slightly in this direction. In one or two isolated cases the feeling was expressed that we should forget most of this research tomfoolery and get back to the three R's of medical education. Herein may lie some of the reasons why we have failed to attract more young men into our field, and indicates that there is a lamentable lowering of standards.

As a possible aid to those departments which need more research funds than are at present available to them, your committee has compiled a list of foundations and other agencies supporting research in the medical sciences, which appears in Appendix II to this report.

Present numbers of graduate students. Forty percent of the 57 departments had no graduate students; the remaining 60% are training 79 persons whose avowed purpose is a career in anatomy. More than half of these students are now in 10 departments. Table 3 shows the distribution of these students by departments.

TABLE 3

NUMBER OF DEPARTMENTS	NUMBER GRADUATE STUDENTS IN EACH	TOTAL
8	1	8
13	2	26
5	3	15
1	4	4
2	5	10
1	6	6
1	10	10
		—
		Grand total 79

The inadequacy of this present number of 79 graduate students may be emphasized by recalling that 107 immediate vacancies now exist in anatomy and 82 additional recruits will be needed to replace losses in the next decade. In addition, 217 extra personnel are thought by the administrative officers of anatomy departments to be needed to bring existing staffs to the level at which teaching would not be excessive and adequate opportunity would be provided for research.

Thus, as closely as your committee can determine, at least 189 individuals have to be recruited into anatomy in the next decade, and a total addition of 406 would be desirable. Of this number, 79, or only 20% of the latter total, are at present in training.

Fellowships for support of graduate students. Graduate students are supported by fellowships as follows:

TABLE 4

NUMBER OF DEPARTMENTS	NUMBER FELLOW- SHIPS IN EACH	TOTAL
4	1	4
14	2	28
5	3	15
3	4	12
1	5	5
2	6	12
1	11	11
		—
		Grand total 87

These fellowships range from \$300.00 to \$3,600 in amount with both the median and the mode of the minimum figure given being \$1,200, or just about the cost of sending the average undergraduate through a year of college if he is careful. It is not without significance that almost half of these fellowships are offered by 7 departments. Your committee had no way of judging whether these departments were the best qualified to train almost half the future supply of anatomists.

As a possible adjunct to existing departmental support of fellowships, your committee has compiled a list of foundations and other agencies supporting fellowships in the medical sciences, which appears in Appendix I to this report. As is pointed out there, however, most of these fellowships are of the postdoctorate type, and so may not be of immediate help in recruiting personnel to our profession.

Teaching facilities in anatomy

Equipment and supplies. Seventy-five percent of the departments indicated that, in the matter of equipment and supplies, the present facilities for teaching anatomy were adequate. In the 25% of departments in which they were not, desired additions in the order of their frequency were as follows:

More space — especially conference rooms or seminar rooms, office and laboratory space for graduate students, research laboratories, animal quarters, embalming room.

More visual education material — especially movies, koda-chrome and other projection slides and projectors, models, museum specimens, charts.

X-ray and fluroscopic equipment.

Special equipment — as band saw, quartz optics, phase microscope, technicon, scopicon, photographic material, deep freeze.

More cadavers. Social agencies of all sorts tend to decrease the supply.

Budget. Sixty percent of the departments indicated that the present annual budget for teaching was adequate. Forty percent of the departments desired annual bugetary increases for teaching, ranging from 5 to 1000%, the mode being 10% and the median being 22%.

Suggestions for improving the profession

The following suggestions, some thoughtful, others petulant, are quoted from the questionnaires, or appended sheets and are, for the sake of convenience, broken down into several categories, namely: salaries, recognition, relations to clinical medicine, teaching, research, sabbatical leaves, scientific visits, travel, journals, books, movies, refresher courses, specialty board, and technical help.

Salaries. "First and foremost: higher salary levels. We believe that the principal reason why young men are prevented from going into anatomy as a career is because of economic necessity. We cannot compete with the practice of medicine and scientific postions in industry."

"The principal need is to attract and *hold* men whose aptitudes and abilities fit them for a career in teaching and research. The crux of this problem, I am convinced, is the provision of adequate salaries. Anatomy shares with other basic medical sciences a competition with the much more remunerative career of medical practice. It would be expecting too much to argue for incomes equal to those from practice; but a fair and just claim can be made for improvement in the salary level."

"Unless the economic status of teachers of anatomy is raised, there will result a still greater shortage than now exists. Many of the most capable well trained younger men who are qualified for clinical practice are going into that field, and replacements are hard to find."

"In this school a number of the clinical staff unknown outside the community, and who engage in practice in addition to their hospital and teaching duties, are paid as much or

more than preclinical men with international reputations in their fields. This is done on the basis of "market value" of the clinical man. Unless administrators are made to realize that the specialist with a Ph.D. degree is as valuable to the institution as the man who has the qualifications to enter practice, high grade men will hesitate to enter the field of teaching and research."

"In schools with full-time clinical men, their salaries tend to be at least twice those of laboratory men."

"Increase salaries so that anatomy as a profession would be more attractive financially."

"Better salaries, security of tenure and retirement benefits."

"Adequate salaries — this is basic."

"The first necessity is greater compensation for services rendered, with financial rewards at the professorial level high enough to furnish incentive and give security and comfort."

"I believe that a budget increase, permitting an increase in the number of personnel and an increase in their salaries, would do more to improve our situation than almost anything else at the present time."

Recognition. "I suggest that steps be taken to acquaint members of the medical profession and teachers in other preclinical fields with the importance of anatomical teaching and the significant contributions to research which anatomists have made and are making. There is a deplorable lack of appreciation for the field of anatomy from the standpoint of teaching and research."

"I think that our self respect and the respect of our colleagues is all that is needed and that this can best be attained by fostering a general recognition of the fact that anatomy is by no means a hand-maid of clinical medicine, but, in its various aspects, is the fount from which flows an appreciable part of the current that keeps medicine out of the hands of witch doctors."

"Better recognition by administrators that anatomy is fundamental to progress in medicine and biology."

"In contrast to others, the profession of anatomy is scarcely known to the informed general public and not in the least understood; and the activities of the anatomist constitute an unthought of and unheard of performance. During the war it was obvious that our government had been well informed of the professional status, training and potential usefulness of the biochemist, physiologist, bacteriologist, pathologist and many others, but the anatomist and his profession remained completely unrecognized."

"Heap a little more adulation upon the profession of anatomy."

"The professor of anatomy should be regarded as important as the professor of surgery."

"The plain fact is, I believe, that anatomy has lost caste with the medical profession as a whole. Once pre-eminent, it now ranks probably at the bottom of the medical sciences. The individuals attracted to teaching openings in anatomy have been largely from biology departments of undergraduate institutions. They have exerted an influence on teaching and research, and have been willing to work on a salary scale lower than it should be. Because of this, anatomy has become compartmentalized and taught as a pure science. In general, anatomists do not speak the same language as the medical men."

Relations to Clinical Medicine. "Make anatomy the basis for departure for every phase of the art of medicine."

"Establish closer liaison with other preclinical sciences and emphasize clinical correlation and application with joint scientific meetings and publicity for basic research in anatomy."

"Suggest realistic attitudes towards relationships of anatomy to clinical specialties. Bring anatomists among the specialties into anatomy departments. General cooperation and collaborative efforts to bring the clinician into contact with the anatomy department."

"Closer contact of anatomists with clinical faculty members in postgraduate work and clinical consultations; attendance at clinical staff meetings and participation in discussion."

“The impossible suggestion that anatomy be returned to being a subject in its own right rather than so much emphasis on the subject as a handmaiden of the “practical aspects” of clinical work.”

“Play up the role of the anatomist as a contributor to basic understanding of every phase of the human body, not a handmaiden to the high and mighty practitioner.”

Teaching. “More stimulating teachers of anatomy are needed. This seems impossible if anatomy is loaded merely with a repetition of classic morphology, which saps the energy and stifles the intellectual potentialities of its teachers. Hours of formal training should be reduced to a minimum and elective courses should be provided for select students to afford maximum of intellectual stimulation for teachers.”

“Anatomy staffs are too small for the teaching responsibilities assumed.”

“Anatomy is too often a “medical school course” and as such a handmaiden to surgery et al. As such, it does not merit academic recognition; it will be relegated to a secondary role and attract persons without exceptional ability to its ranks.”

“Indicate more interest and give more assistance in solving problems confronting the student of clinical medicine.”

“Revise our teaching methods; make gross anatomy more functional instead of memorization of minutia. Microscopic anatomy is forced by its very nature to be more functional. This approach would improve the profession in the sense that our teaching would be less deadening.”

“Lowering of individual teaching loads by increasing academic staff to allow time for research.”

“During the past thirty years, anatomy has suffered a tremendous reduction in hours in the curriculum. I believe the association should come forward with a strong resolution clarifying the importance of a thorough training in anatomy as a prerequisite for the training which follows and for proficient practice of medicine. I am wondering if anatomists, in their enthusiasm for research, have not been too willing and cooperative in giving up this time.”

"Lighter loads in routine teaching are suggested to permit increased facilities for training graduate students, elective courses and more free time for research."

"Teachers should be trained in clinical fields and adjust emphasis accordingly for medical students."

"The teaching load should be stabilized at not more than 200 hours a year."

"Lighter teaching loads in the majority of medical schools. The hours of instruction in anatomy far exceed those allotted to other departments."

Research. "Termination of administrative attitude that research funds should be provided by grants from foundations or government departments and provision of adequate research budgets."

"Make grants for research more in the nature of an endowment."

"More teaching and research from the medical viewpoint."

"Research content should be deepened and broadened to include modern cellular biology, studied by physical and chemical means."

"A department research budget, exclusive of outside grants, of an unrestricted nature, should be established. This need not be large but should guarantee a minimum of freedom of research to all staff members."

"Posts for senior men that offer adequate salaries, without attached administrative duties, and the opportunity to carry out research studies throughout the year, even during the time when teaching duties were heaviest. To such posts young men would aim; older men should utilize them as opportunities."

Sabbatical Leaves, Scientific Visits, Travel, etc. "Re-institution of sabbatical leaves and short leaves of absence with pay for the purpose of visits to other institutions."

"Restore sabbaticals on pay so that staff members, especially younger ones, can spend periods in other laboratories. Inaugurate arrangements between schools to effectuate this, possibly on an exchange basis."

“An interchange of visiting staff members is suggested, particularly at the lower rank levels (instructor or assistant professor).”

“Establish exchange professorships for the distribution of educational ideas.”

“More leaves of absence with salary for study where promising new fields of research and techniques have been developed.”

“We need more travel money to enable the younger men to go to meetings and to spend summers or semesters at research centers.”

“Establish an anatomical Woods Hole.”

“Establish adequate budgetary provision to pay expenses to annual and other scientific meetings, attendance at which promotes the exchange of ideas that is most stimulating for research and teaching.”

“Travel funds for meetings and scientific visits.”

“Have a system of exchange so that younger men in smaller schools could teach a semester in one of the larger schools and come in contact with research going on in those places.”

Journals, Books, Movies, Refresher Courses, Specialty Board. “Summarizing papers in the field of clinical anatomy written by experts on invitation of editor for publication in anatomical journals.”

“Serial publication of something along the line of Annual Review of Physiology or Physiological Reviews, containing comprehensive summaries of the field.”

“Publication of a more comprehensible textbook in gross anatomy for students, along the lines of modern neuroanatomy texts with elimination of histological, embryological and neuroanatomical material.”

“Publication of an encyclopedic work consisting of a number of volumes for reference purposes and including all fields of anatomy.”

“A functional anatomy textbook, half the size of our present tomes, is needed.”

“Publication of movies for teaching purposes, using modern means of visual education, such as animated cartoons, sound and color, particularly for use in gross anatomy, neuroanatomy and embryology. This project to be undertaken by an endowed organization with anatomists and movie experts serving as consultants.”

“Introduction of refresher courses at our annual meetings comparable to similar courses given at clinical meetings.”

“It may be forced upon us to have a standardization board. Our membership requirements are good. Maybe they should be increased.”

“Establishment of a Specialty Board would greatly improve the profession. Certification should not be based on examinations, but on training, teaching experience, research, attendance at meetings and participation in the program of these meetings. A certification of character or indication of the esteem with which fellow anatomists regard the individual should also be required. Medical schools should appreciate and realize the merit of selecting a person with such approval or certification.”

Technical Help. “Administrative work in the department should be delegated to an administrative assistant, who need not be a professional anatomist.”

“Faculty participation in routine committee work should be reduced.”

“A minimum of one person of technical or secretarial rank for each staff member of instructor grade or higher.”

“Improvement in working conditions by increase in the technical and secretarial staff.”

“Improvement in equipment and increase in staff for photographic laboratories.”

“Establishment of machine shops within medical schools.”

Suggestions for attracting young men to anatomy

These fell into three categories: publicity, fellowship program and standards. The following are direct quotations from the questionnaires received by your committee:

Publicity. "Opportunities for fellowship training programs, which pay a living wage with sufficient spread of information concerning them to attract young people with scientific qualifications."

"An educational program in the Universities pointing out attractive features of a career in anatomy which, even now, offers more research time and lighter teaching loads than other biological sciences. I do not think that university students are familiar with these facts, for there are far more graduate students in other fields of biology than in anatomy."

"Some propaganda in liberal arts colleges would help. Pre-medical advisers are often unaware of opportunities in the fields of anatomy and physiology at the medical school level. They advise their best students to go into medicine or surgery — or zoology, chemistry or physics."

"Publicity as to the possibilities in graduate work in anatomy as compared with zoology, psychology, etc. Increased number of assistantships with disseminated information about these opportunities would help."

"The available young men of our medical and graduate classes should be presented with the facts and opportunities of our profession. We are sure that we lose many young men because they are unaware of the real nature of academic life. Such ignorance is in great part due to failure of the faculty to dispell it."

"Certainly some percentage of young men in medicine are definitely interested in basic science teaching and research, but, surprising as it may seem, remain entirely ignorant of the opportunities to be obtained in these fields. Attesting to this is the fact that in every instance where a medically trained man has assisted in anatomy, he became impressed with further opportunities in the field. Certainly not all medical graduates are so keenly interested in practice as to ignore opportunities in allied teaching fields."

"Drug companies, governmental agenices and research institutes entice numbers of the medical profession into their organizations by pursuing an active advertising campaign.

Is it not possible that medical schools, or affiliated societies, could likewise pursue some type of an educational policy that would acquaint interested medical personnel with opportunities in basic science?"

"Make the profession seem more attractive to young men. To many possible students the true value of cellular studies and the scope of the field is unknown. Anatomy connotes 'gross anatomy' and a long dead subject."

Fellowship Programs. "Fellowships with a stipend above the pre-war starvation level so that students with little or no financial backing can, without worry, immerse themselves in study and research. It is suggested that such fellowships be freed of a teaching requirement."

"Joint teaching and research fellowships for graduate and medical students. It may be that more medical students and fewer graduate students will be available for posts in anatomy. If so, this means that provisions for the scientifically minded medical student might well include: (1) financial aid for one to two years, in addition to course work in medical school; (2) original studies in research and not the conduct of technical studies or aspects of the work of staff members; (3) exposure to seminars, luncheon meetings with the staff, visits to other laboratories at other schools to broaden the experience of the students. These fellowships must be sufficiently well financed that the best students may be selected regardless of their ability to pay their own way."

"Establish fellowships carrying an annual stipend of \$2,000 or more. Emphasize that in working for a doctorate it is possible to earn while you learn. Make it clear that positions in anatomy do offer opportunities for gratifying intellectual curiosity and research. Point out that attempts are being made to improve anatomy as a profession."

It is practically imperative that the training of anatomists be delegated to the large state and privately endowed medical schools. Wherever practicable, these schools should undertake a program which would lead to a constant supply of young anatomists. The ideal situation would be to have a

large department of anatomy set up as a pyramid with a broad base, made up of many teaching fellows, staggered so that there would be an overlapping of three, two, and one year men, or four year men if the program were longer. These fellows should be held responsible for all phases of anatomy: gross, histology, embryology and neuroanatomy, as far as the subject matter is concerned, and they should have teaching experience in at least two or three of these disciplines, if not all. Research should not necessarily be restricted to the rigid boundaries of anatomy, but might well overlap into other fields, though it should have an anatomical background. It should be realized that in addition to teaching of anatomical subjects and research a certain amount of course work in advanced anatomy ought to be an absolute requirement. As many courses as possible in the first two years of the medical curriculum should also be taken. This might necessitate a four year program, which, indeed, is not unreasonable."

"It seems to me very desirable that especially promising young men, of whom we see one or two almost every year, be given the chance to drop out of the regular course for a year or more at a time, when their interest is really kindled, and be given an opportunity to see what teaching and research in a department of anatomy is like, without being penalized financially or otherwise. Such a fellowship as is contemplated would carry no obligation to continue in anatomy, but would provide the opportunity to see whether the student would care for a career in this field. If he did not, he would not be harmed as a clinician, and if he did, the rest of his course would be an even more valuable preparation for his subsequent teaching."

"Offer more assistantships to selected students who appear to have an interest in teaching after they have completed the first or second year of medicine. Such an assistantship should enable the student to take part time medical work and assist in the laboratory work and give some lectures in the various anatomy courses. A sufficient salary should be paid to enable the student to complete his medical education without further

cost to him and should provide his living expenses. He would be considered a member of the anatomy staff, participate in seminars and carry on research activities. It would take several years longer for him to obtain an M.D. degree, but he would receive it without additional cost and would be thoroughly trained and indoctrinated in the teaching of anatomy. In most cases these men would become full-time teachers of anatomy. The Ph.D. degree would not be necessary."

"Try to develop promising medical students who will come back to the laboratory after graduation with primary interest in research, but lively interest in clinical problems. We are doing satisfactorily in this respect at present because of active physiological research programs of sufficient variety to ensnare the interest of medical and graduate students, and provisions for opportunity and aid in getting them started by the end of, or even during the freshman year in research programs. We find it possible to compete with other departments on at least equal footing by providing early outlet for investigative tendencies and by simply being alert to detect such tendencies. This would not be possible, I am sure, if our main emphasis were on descriptive morphology, nor if we took too belligerent an anti-clinical view."

"Provide more fellowships, up to \$2,000 a year, to carry a young man, and, if necessary his family, through the three or four years of graduate work and training in anatomy."

"Establish scholarships that pay a salary on which people can live while they are in training."

Standards. "Careful selection of graduate students. We accept graduate students on probation. They take the full load of the first semester medical courses. If they are outstanding and can earn grades of "A" in the upper fifth of the class, we allow them to continue. If they are below this level we advise them to seek other careers. We believe there are too many people in anatomy and allied fields who are mediocre and who should have been screened out at the start. The offer of subsidies to graduate students would help only if we are willing to screen the recipients of such funds ruthlessly."

“Another approach to this problem is to set an example of what good teaching and research can be and to make the student aware of the satisfaction in being a professor.”

“Anatomists should be good enough to create in young men the desire to emulate them and to select anatomy as a career.”

“Make teaching as a profession more attractive by increasing the dignity of academic positions. Attention should be paid not only to material factors, such as remuneration, tenure and pension, but also to the intangibles as expressed in faculty participation in the running of departments and the institution as a whole.”

“There should be a radical change in the point of view of younger men that the world owes them a living of a high order, even if they wish to do scholarly work. Too many men fail to believe that some sacrifice in income might be in order if they are permitted to do what they really wish to do.”

Conclusion

The foregoing comments indicate to your committee that members of this profession are generally aware of the serious situation which at present exists in anatomy in this country. If there be any who are not, it feels that this survey should adequately alert them.

While a number of valuable suggestions have been made, your committee feels that a comprehensive series of proposals for improving the present situation is of such basic importance for the maintenance of anatomy as a profession, that another year's study should be devoted to its preparation.

We suggest, to this end, that our President appoint a nine member committee, consisting of three persons representing gross anatomy, three histology, two neuroanatomy and one embryology, who should consult with other members of their specialties and with representatives of the branches of medicine and surgery and of biology, with the objective of preparing a report concerned with:

1. Coordinating the interests of anatomy with those of clinical medicine on the one hand, and of biology on the other.

2. Formulating proposals concerning the role of anatomy in medical education.

3. Characterizing major trends in the research interests of anatomists and suggesting means of furthering them; and, most important of all,

4. Planning an effective program for the recruitment and training of new personnel to fill our dwindling ranks.

LOUIS B. FLEXNER

WM. W. GREULICH

H. W. MAGOUN

GORDON H. SCOTT

WILLIAM L. STRAUS

Summary

1. On the basis of the 1948 list of members of the Association, there are 139 full professors, 102 associate professors, 87 assistant professors, and 20 instructors in departments of anatomy of medical schools. These data are alarming for they indicate that no one of the lower ranks is large enough to replace the rank above it.

2. Fifty-five professorships will become vacant from retirement alone during the next 10 years. If no more than a one-to-one ratio of replacements is advanced, at least 55 young men should be recruited into the profession during this 10-year period. To maintain the profession at its present status with a replacement ratio of three in subprofessorial to two in professorial ranks, 82 young men should be recruited during the period.

3. Fifty-seven questionnaires or 70% of those mailed out were returned with answers.

4. From the questionnaires it was found that there are places now vacant for 107 persons with various degrees of anatomical training. These are divided as follows: 5 professors, 8 associate professors, 36 assistant professors, and 58 instructors. Anatomists believed that 217 added personnel (62% more than the present level) are needed to bring their staffs to a level where teaching will not be excessive and at which adequate opportunity will be provided for research.

5. Present annual salaries for instructors range from \$1,500 to \$5,000; for assistant professors, from \$2,500 to \$6,200; for associate professors, from \$4,000 to \$10,000; and for professors, from \$4,000 to \$15,000.

6. Increases in salary stated to be desirable are analyzed in the body of the report.

7. The research funds expended by the 57 anatomy departments furnishing data totaled only \$679,180, of which 14% or \$96,500 came from departmental appropriations and the remaining 86% from outside sources. Thirty percent of these departments have no research appropriations from the University budget, and 25% of the departments receive no funds from sources outside the school.

8. Forty percent of the 57 departments have no graduate students; the remaining 60% were training 79 persons whose avowed purpose is a career in anatomy. These students received fellowships ranging from \$300 to \$3,600.

9. *Remarks on teaching facilities contained in answers to questionnaires.* These are regarded as adequate in 75% of the departments. In the other 25%, desired addition, in order of frequency, relate to space, visual-education material, equipment, and cadavers. Forty percent of the departments desire annual budgetary increases for teaching.

10. *Suggestions for improving the profession contained in answers to questionnaires.* (a) Larger salaries with which to attract and hold young men equipped for a career in anatomy. (b) An attempt to secure greater recognition from the other preclinical and the clinical sciences of the contribution made to medical teaching and research by anatomy. (c) Modification of present teaching methods by introduction of functional and clinical concepts, reduction of individual teaching loads, and production of better textbooks of gross anatomy. (d) Greater support and more time for research, and the broadening of research interests. (e) Increase of facilities for travel and study at other research and teaching centers, especially for younger staff members. (f) More technical and secretarial assistance. (g) Establishment of adequate fellow-

ship programs to attract good young men to careers in anatomy.

11. *Lists of available fellowships and sources of grants-in-aid are given in the body of the report.*

APPENDIX I

Fellowship Sources

The National Research Council (Division of Medical Sciences, 2101 Constitution Ave., Washington 25, D.C.) administers the following fellowships open to young anatomists with the M.D. or Ph.D. degree:

Fellowships in the Medical Sciences established by the Rockefeller Foundation to provide opportunity for research training for recent graduates who wish to devote themselves to an investigative career in the medical sciences.

Merck Fellowships open to candidates with training in chemistry or biology, including the preclinical medical sciences, who have demonstrated unusual talent for experimental research.

American Cancer Society Fellowships open to candidates in any of the many basic sciences which may contribute to the advancement of knowledge in the field of neoplastic disease.

Atomic Energy Commission Pre- and Postdoctoral Fellowships in any of the biological sciences which, in a broad sense, are basic to atomic research or associated with the development of atomic energy or its by-products.

Fellowships in Poliomyelitis and Related Fields established by the National Foundation for Infantile Paralysis to provide special training and experience in the study of virus diseases and in those basic medical sciences of particular value in furthering progress in orthopedic surgery.

Fellowships in Fields Related to Arthritis and Rheumatism. Details to be announced.

The National Institute of Health Research Fellowships (Division of Research Grants and Fellowships, National Institute of Health, Bethesda 14, Maryland) are awarded to individuals who have had postgraduate work in the various fields of medical and related sciences, and are of two types:

Junior Research Fellowships are available to individuals holding the master's degree or who have completed an equivalent amount of graduate study.

Senior Research Fellowships available to individuals holding a doctorate degree.

The Markle Foundation (14 Wall St., New York 5, N. Y.) has established a program of 5-year grants for Scholars in Medical Science, intended to aid young men and women planning careers in academic medicine, who hold a full-time faculty appointment on the staff of a medical school, but who have not yet attained a scientific reputation sufficient to attract financial support from foundations or other sources. Each school is invited to submit the name of one candidate.

The John Simon Guggenheim Memorial Foundation (551 Fifth Ave., New York 17, N. Y.) awards *Fellowships for Research* intended for men and women of high intellectual and personal qualifications who have already demonstrated unusual capacity for productive scholarship.

The Commonwealth Fund (41 East 57th St., New York 22, N. Y.) awards fellowships to individuals of proven ability.

The Life Insurance Medical Research Fund (2 East 103rd St., New York 29, N. Y.) supports postdoctorate (M.D. or Ph.D.) fellowships for training in research in the broad field of cardiovascular function or disease.

The American Heart Association (1775 Broadway, New York 19, N. Y.) supports advanced fellowships for research within the broad field of diseases of the heart and blood vessels.

Yale University School of Medicine (333 Cedar St., New Haven, Conn.) administers three funds which support Fellowships, both at Yale and elsewhere, in fields related to cancer. These are:

The Anna Fuller Fund (205 Church St., New Haven, Conn.)

The Jane Coffin Childs Memorial Fund for Medical Research supports fellows at any school or institution who are interested in abnormal growth.

The James Hudson Brown Memorial Fund supports fellowships at both junior and senior levels.

In considering the above, it is obvious that the majority of fellowships available at present are offered to individuals possessing an M.D. or Ph.D. degree. Since such possibilities for postdoctorate assistance exist, it would seem the course of present wisdom for anatomy departments to concentrate upon encouraging as many capable individuals as possible to complete their doctorate programs, and to utilize all available departmental resources to that end. Once the doctorate is in sight or obtained, it would then seem wise for the department to do everything possible to aid the individual in obtaining fellowship assistance from one or another of the above sources.

These postdoctorate fellowships are of two general types. Certain of those administered by the National Research Council and the

National Institute of Health are unrestricted as to fields of interest. Others are designed to train individuals in specific fields of research related to prevalent diseases, as cancer, cardio-vascular disorders, poliomyelitis, arthritis and rheumatism, etc.

APPENDIX II

Grant sources

In 1940, the most recent year for which an analysis of annual foundation aid is available,¹ a total of \$12,000,000 was contributed to the field of medicine and public health; \$650,000 of this sum going to the support of research in the medical sciences. In that year, two grants were made in anatomy, together amounting to \$6,000. In 1940, therefore, anatomy received slightly less than 1% of the foundation support given to the medical sciences, and about 0.05% of that received by the field of medicine and public health in general.

Though the present situation is considerably improved, the following list of foundations and other agencies aiding investigation in the medical sciences may, nevertheless, be useful:

The National Research Council, through special committees, handles research funds as follows:

The Committee on Growth administers research funds for the American Cancer Society, available to anatomists investigating problems of growth.

The Committee on Research in Endocrinology supports work in the general fields of experimental and clinical endocrinology, as well as studies of the effect of sex hormones on nonsexual functions.

The Committee for Research in Problems of Sex, and

The Committee on Human Reproduction have funds in more limited amounts available.

The Food and Nutrition Board has recently published a Survey of Food and Nutrition Research, section II of which lists organizations supporting research in this field.

The U. S. Public Health Service (Division of Research Grants and Fellowships, National Institute of Health, Bethesda 14, Maryland) supports a grant program with the following major objectives: (1) to expand research activities in universities and other institutions; (2) to stimulate the initiation of research in small colleges where previous research programs have been limited or non-existent.

¹Raymond Rich and Associates: American Foundations and their Fields, N. Y., 1942.

ent; (3) to encourage investigators to undertake research in neglected fields; and (4) to provide training for scientific personnel. A comprehensive exposition of the program is to be found in Science 104: 559, 1946. Applications are passed upon by an appropriate study section composed of experts, largely from universities, in the field in which the proposed research lies.

The Office of Naval Research (Department of the Navy, Washington 25, D. C.) encourages and gives broad support to both scientific research and the development of research manpower by contracts with universities and other institutions. It is concerned mainly with the support of basic research and offers the greatest possible freedom to scientists in investigation in all branches of the medical sciences, including anatomy.

The Atomic Energy Commission (Washington, D. C.) is conducting a research program in the fields of Biology, Medicine and Applied Biophysics, and is supporting research in these fields at a number of non-profit Institutions, Hospitals and Universities through research contracts. The Commission expects to support additional projects in such unclassified areas as the following: (1) experiments on the fundamental action of radiation on living matter; (2) the study of physical methods for measurements of dosage of radioactivity to the body; (3) selected studies on the use of isotopes and radiation in treatment of disease; and, (4) studies in the mechanism, diagnosis and treatment of radiation damage in humans.

Proposals need not be submitted on any standard form, but they should be completely presented and properly endorsed. Proposals should be submitted to the Director, Division of Biology and Medicine, U. S. Atomic Energy Commission, Washington, D. C. Proposals are reviewed by, and, if approved for support, will be conducted under general technical supervision of the Washington Staff. Negotiation of contracts and business aspects of contracts will ordinarily be carried out by the nearest Commission Operations Office.

Following is a partial list of private foundations supporting research in the medical sciences, fields of special interest being indicated whenever they are known to exist. For further information write directly to:

American Foundation for High Blood Pressure (705 Marian Bldg., Cleveland 13, Ohio) provides grants for research in hypertension, arteriosclerosis and associated ailments.

American Heart Association provides grants for research within the broad field of diseases of the heart and blood vessels. Recommendations of the Research Policy Committee are to be found in the *American Heart Journal*, 36: 463, 1948.

Arthritis and Rheumatism Foundation (535 Fifth Ave., New York 17, N. Y.) supports research in the basic medical sciences contributory to knowledge of arthritis and rheumatism.

The Commonwealth Foundation (41 East 57th St., New York 22, N. Y.)

The Dazian Foundation for Medical Research (142 W. 44th St., New York 18, N. Y.)

The Samuel S. Fels Foundation (1315 Walnut St., Philadelphia 7, Pennsylvania)

The Life Insurance Medical Research Fund makes available grants for fundamental research which bears upon cardiovascular problems, as well as for clinical investigation in this field.

The Lucius N. Litauer Foundation (235 Fourth Ave., New York, N. Y.)

The Josiah Macy Jr. Foundation (565 Park Ave., New York 21, N. Y.)

The National Foundation for Infantile Paralysis (120 Broadway, New York 5, N. Y.) awards grants for research contributory to the cause, nature and methods of prevention of poliomyelitis and to the amelioration of its harmful after-effects.

The Ella Sachs Plotz Foundation (30 Pine St., New York 5, N. Y.)

The Rockefeller Foundation (49 West 49th St., New York 20, N. Y.)

The Viking Fund (14 East 71st St., New York 21, N. Y.) awards grants for research in anthropology and related fields.

Certain of the agencies and foundations listed above, as the U. S. Public Health Service, the Office of Naval Research, and some of the private foundations, support research in such a wide variety of fields as to be virtually unrestricted in their interests. Others administer funds promoting investigation within a given field, as the Committee on Growth or the Committee on Endocrinology of the National Research Council. Still others limit their support to projects which promise to contribute to the treatment or prevention of a specific disease, as the National Foundation for Infantile Paralysis, the Life Insurance Fund for Medical Research, or the Arthritis and Rheumatism Foundation. In connection with this latter group of foundations, it should be pointed out that they recognize the value of contributions to basic problems associated with the disease in question, and are glad to support fundamental research providing its relation to their clinical objectives are made clear.

BANQUET ADDRESS BY MARION HINES

American Association of Anatomists

Philadelphia — April 14, 1949

Ten years ago the Council on Medical Education, appointed by the American Medical Association reported upon the state of Anatomy in the 66 4-year medical schools in Continental United States. In the Anatomy Departments of about 30 schools they found no evidence of scientific interest. In those of the remaining 36, they wrote that the effect of an active research program could be "actually sensed." When given proper student-teacher relationships, research had a definite stimulating effect on students. Further, they noted that in the 10 first ranking departments (and no names were mentioned) the number of hours allocated Gross Anatomy was the lowest and in the 10 lowest ranking departments, the number of hours given that subject was the highest. In the former group Gross Anatomy was confined to one semester or to two trimesters, in the latter, it was spread over the whole first year or more. All of the higher ranking departments used living and fresh tissues for study and in some, experimental methods formed a part of the material presented. There was a definite trend away from a static to a dynamic conception of Anatomy in the majority of all medical schools.

The Council did not recommend any attempt to bring about uniformity in the courses of Anatomy taught in our medical schools, for they wrote "standards should deal not with detail of course content, clock hours, or methods, but rather with those fundamental principles which are essential to any or every course in Anatomy." The Council neglected to state those fundamental principles; they left the reader to gather

from their comments what they might be. In the 10 highest ranking departments, suitable space and equipment for teaching and research were found. In 9 of these 10, the budget was in excess of the median (\$273.00) per student and the teacher-student ratio varied between 1:6 to 1:10. Living and fresh tissues were used in both gross and microscopic Anatomy and research was in progress. Further, although they recommended that teachers in medical schools be doctors of medicine, nonetheless, they noted that some of the best teachers of Anatomy had only the scholar's degree.

The fundamental principles left for conjecture seemed to be that good teaching was not related to content of a course as such, to clock hours, to methods, or to the type of doctors degree held by the members of the faculty. Rather, it was the inquisitive and creative type of mind within the Anatomy faculties, which made Anatomy a living, breathing subject.

The decade before 1940 witnessed a revolution in college education, a reassessment of needs and of values. This reassessment recognized on the one hand the need of pointing some of undergraduate education toward vocational training and on the other the value of a new orientation in the old education, one, which would use the knowledge of the past to analyze and define the good for the present. Such a scholastic preparation for living and working is flexible and resilient enough for use in an age of transition. There has been no comparable revolution in medical education. Although in certain schools, public health and preventive medicine have been added to an already crowded curriculum, there has been no great and radical change in the curriculum as such, nor in the concepts of basic medical education. And this in a state which is demanding greater social service from its medical profession! Only too frequently curriculum committees sit and juggle the allocation of hours, giving fewer hours to subjects taught by those who do not protest and more hours to those taught by a faculty which does protest. It is rare, indeed, to find a curriculum committee which discusses the type of doctor they wish their school to

produce and then considers how the curriculum can be created to produce the valued product. It is passing strange that the relative growth in preclinical sciences has not caused a re-evaluation of their specific place in medical education. Indeed, in some medical schools the development of the teaching of preclinical sciences is made subordinate to the demands of the teachers of clinical subjects. There has been no universal or general attempt to evaluate method, to state fundamental principles or to discuss relative values in the education of doctors. Instead, we hear of the need for more doctors, not of more and differently trained doctors. We hear a great deal of the *cost* of medical care and not enough of the social orientation of the physician. Five thousand new doctors per year is not enough. Of those 5,000 who will receive the M. D. degree in June, how many will be cognizant 20 years from now of the advance and re-evaluations, inevitable in medicine? And they will practice medicine for twice that time.

We speak of the art and science of the practice of medicine. The phrase itself implies a broad and liberal education as well as a scientific education leading toward the profession. There is no antagonism in medicine between a vocational and a liberal education, although the present required prerequisites neglect those social and economic subjects which might prepare the profession for a more adequate understanding of the environmental factors which influence the individuals who seek a return to health. Nor do these prerequisites include subjects which suggest the relation of health of the body politic to the types of soils on which their food grows. Deficiency diseases may not be the result of inadequate intake, but may rather be related to deficiencies in the soil itself. Subclinical vitamin deficiencies are extraordinarily common. Few doctors in practice for 20 years or more and even less take heed of this problem. Indeed, more than 75% of the 1948 report of the Division of Biology and Agriculture of the National Research Council (to which we as the American Association of Anatomists belong) is given to the prob-

lems of food and nutrition not only of man but also of domestic animals.

A man, or a woman for that matter, assumes many guises during each day. He is a producer, a consumer, a citizen, a critic, of government and of other peoples' way of living, and at present, a potential soldier, a teacher to someone, a student of another, as well as a son, a father, a brother, and a husband. He lives through each day amid an amazing complexity of human relationships. If, however, he is a doctor of medicine, this normal daily complexity of human relationships is multiplied many times for him, and the awareness of a similar complexity in the daily life of each of his patients is expected of him by them. No subject required as entrance to a medical school offers him any understanding of these relationships. Only a few first year students in medicine, greater, however, now than ever before recognize the necessity of such an understanding. For many of them the first course in psychiatry is their introduction to the science of human interaction. And yet a liberal education which includes a study of literature and of history might increase their sensitiveness to human beings and prepare them for an understanding of the man or of the woman who has the disease and its concomitant pain which constrains them to call a doctor.

Being a doctor is, indeed, a full time job, requiring pre-eminantly a useable knowledge of as much of the field of medicine as he can encompass, a cultural perspective and social orientation as well as imagination, "that sort of logic" which for the few is creative, and which for the more could be interpretive and which for all should be sympathetic.

Each year brings a new 5,000 young men and young women into the medical schools of this country, men and women selected by admission committees from among those many thousands who desire to enter this peculiarly engrossing profession. The majority of these candidates have passed the medical aptitude test. Although this test seems to give them

fair assurance of graduation in medicine, it is incapable of giving the public any reassurance that the new made doctor will continue to grow in wisdom and in the knowledge of an expanding profession. Medical aptitude tests cannot compete with those used by the Army in the selection of pilots for the Air Corps; for those tests are able to predict that the candidates with scores in the lowest category will not graduate from flying training.

In 1940 I discovered in my first year class a young woman who had chosen medicine after she was given a high rating in the aptitude tests for medicine and law. Although she did good work throughout the 4 years, medicine never held for her the peculiar engrossing interest which springs from an inner inescapable choice. In the next year, I came upon a young man, whose questions in histology were those which a good mind asks about unfamiliar material. For two months I answered his questions in great detail and then asked one myself.

“What was your major at Harvard?”

He looked up, a little startled and smiled slowly, “I hate to tell you, Dr. Hines.” Encouraged by my silent interest, he continued, “I majored in the History of Art, not Occidental but Oriental Art.”

“What made you choose Medicine, then?”

“I do not know,” he said. “In my senior year, I suddenly wanted to be a doctor. The summer after I graduated I began to prepare for medicine. I took the medical aptitude test and failed. Johns Hopkins was the only medical school which would take me. The Dean of one school has placed a bet of \$25.00 that I would fail the first year.” He looked up brightly, “Do you think I shall fail, Dr. Hines?”

“No,” I replied, “I do not think so.”

And I did not. Indeed, I never had a worry about that young man. At the end of his first year he passed all subjects and was not warned in spite of the majority of D's on his record.

In his second and third years, he was given B's and C's, and in his 4th A's and B's. He was given the second choice of internships in Medicine at the Johns Hopkins Hospital and sent thereafter to Germany with the Army of Occupation. In one of his letters written me from Southern Germany, I found these two sentences.

"Never in my life will I again have the opportunity to examine so many normal young men. Already, I have listened to more than 1,200 normal hearts and lungs."

The young man is already an exceptional doctor. He was not idle even when he had no "interesting cases." Would that there were an infallible test for professional growth and intellectual maturation!

Scientists in medicine are not gods, rather they are men and limited as all men are. Our students are men and sometimes women. Indeed they are human, with limitations and qualities which are aids or hindrances in the practice of their profession. No education whatsoever seems capable of transforming character or personality. But, these 4 years of training in medicine can lead medical students to a maturity which is able to meet a growing profession and a changing world with youthful anticipation.

The type of doctors who will hold in their hands the health and well being of the people of this country in 1969 will be created in large part by the teachers of Anatomy in our Medical Schools in 1949. The new student meets the faculty of Anatomy first, when he is most impressionable. These new students sense our attitude toward our work, our teachings, and especially toward themselves as human beings. They feel the depth of our experience and the height of our dedication. Whether we like it or not, we give them their early orientation not only toward the subjects we teach but also toward the whole of Medicine. We, as Anatomists, have therefore a serious responsibility to the young men and young women we introduce to the study of their chosen profession. In the future, lives may be transformed or perhaps seriously injured by the success or by the failure of these young people.

If they leave us without learning that the body of knowledge taught in medical schools is not static, but rather dynamic that it grows and changes, they will fail as doctors, no matter how well they do with collections. If they have not learned before the end of the first year that their education is only begun when they can write M.D. after their names, that it is only begun when their training in hospitals is finished, indeed, if they do not learn that they can never settle down to the practice of medicine, then we, as teachers, will have failed in our responsibility. And they learn by example not precept. These students are not only practitioners of medicine to be, they are more. They are men and women, and as men and women, they must orient themselves in a society which is changing, a society which is in the process of a revolution; for, no one can foretell its state of being even a few years hence. The future toward which these young people look is certainly uncertain; the time now calls for a re-evaluation of all values, redefinition of the faith by which we live. As Sir Richard Livingstone says, this is a time "for the adventurous, the romantic, and the courageous." *This* is then a time for the young, who are not weighed down by the baggage of experience. Can we, as we teach Anatomy, the oldest subject in the Medical curriculum, prepare them for this time?

The program at this meeting is witness to the absorbing interest (whatever the motive) which has gripped the American Anatomy faculties, in exploring the unknown. To maintain and nourish that interest in research should be the purpose of the head of each department of Anatomy. Although the nourishment for that interest springs from a human tolerance and a living intellectual curiosity, the material for research must, at least, be adequately provided by the administration. If that curiosity is to continue it must imbue a new generation. The potential new generation exists in the first year students of medicine for they are to become the new generation of Anatomists and the new generation of doctors. Thus teaching has two branches, the training of students as future doctors and the training of a few of those

students as future Anatomists. These two branches share a common trunk with research and both are rooted in intellectual curiosity. The maintenance of teaching and research in bilateral symmetry needs care and nourishment. The young individual on these faculties soon learns that research appears to be the stronger branch, the one he must climb to secure the fruit of advancement. And yet a man is not gauged only by the number of published papers (for some people can do more than count) but also by his ability to stimulate the curiosity of younger young men.

Teaching and research can function in a bilateral symmetrical growth only when the methods of research are those of teaching. Thus, teaching is incorporated in the curiosity which creates research, and becomes a living, breathing activity. No subject is presented to the air. Rather, it is taught to young men and young women. Each group is different. That which is learned from one group may not work with another. Experience is always utilized a year too late, and students are not litter mates. There is no real mean, no real control. Nonetheless, after more than three decades of teaching in three institutions, in three very different environments, with students of heterogenous backgrounds and abilities, I have found that they share three characteristics. They are young; they are idealist; they are hungry. It is easy to present to the young, to the idealist, to the hungry, the tissue of which they, themselves are composed. It is delightful to lead them to consider the activity of the master tissue which controls living processes, by adapting its presentation to these three common characteristics.

Students are young, intellectually and emotionally, although they have reached the physical and legal status of an adult. Many of them have not outgrown the vested authority of the textbook. These prefer the textbook to the material presented. They assume that all is known, for they have not outgrown their devotion to words on paper. It is rare, however, to discover that a small textbook is preferred to a larger one, for the simple reason they cannot "memorize such a

big book," as I was frankly told by a young woman in Chicago, 32 years ago. Many of them desire to please the teacher, not to begin to think about the subject. Indeed some are mere children.

To the child, the book is magic. Children learn quickly that the adult can look at a book, turn the pages *and* a story unfolds. The story becomes identified with the very words themselves. Not a word can be changed, for the words *are* the story and the story is an enlargement of life as they know it outside the book. The words are the magic. In normal development this rigid conformation to the very words and very phrases of the tale is followed by an heroic age when they control the story and its magic and thereby escape in fantasy to a life not limited by their small physical powers. Before they go to school they become willing to listen to a story in which their daily life is made romantic and they in turn become the actors. And when they learn to read, they have become independent investigators of adventure, and of romance. In a sense a medical education recapitulates for the very young student this childish growth from dependence to independence.

They are idealistic, holding as true a rigid idealism which demands conformity of all investigators and of all teachers. Nothing human is as perfect as they have concluded it has *got* to be. You and I know that many investigators, scientists, and teachers care more for themselves than their subjects, that they would rather be right than discover what is true. You and I know that there are others who are devoted, even dedicated to their work, who have respect for that work and faith in the validity of their own sense perceptions, who are moved by reason and by the vision of truth, and who have faith in the potentialities of young people. Students are quick to sense and criticize the former group and unless the latter is held before them their idealism is blackened. Only out of *his* experience will the student be able to re-create his idealism. If this re-creation does not occur, we, as teachers, are responsible.

They are hungry, yes, hungry, even for food. I know. I have fed them. I have never met a medical student who refused a second helping. They are hungry to grow up; to be allowed intellectual companionship with their teachers. They are hungry for a chance to think their own thoughts, to test their own ideas. They are hungry for the evidence of generosity on our part, for evidence that their own faith in themselves is justified. They are hungry, even, to destroy their own idealist rigidity. There are those among them who wish to dedicate their energies to the increase of knowledge in some subject.

I believe that it is possible to present the subjects of Anatomy, especially those with which I have intimate experience, microscopic Anatomy and Neurology, so that the immature student begins to grow up, and the more mature continues the process of maturation, so that rigid idealism is re-created as tolerate understanding and that the hungry are adequately fed.

The mature mind is capable of being objective; for such a mind has so much respect for the object which it examines that it can afford to see it as it is. Historically, the nature of an object investigated has changed with the ability of observers to see its component parts and its relation to the whole of which it is a part. The history of techniques used in Microscopic Anatomy is a history of methods for the extension of vision. There is a direct interrelatedness between the development of the microscope, of the microtome, of methods of fixation and of staining and the changing concepts of the nature of living tissue. The analysis of anatomical relations of nerve cells, for example, is dependent not only upon the cytological and histological changes secondary to injury but also upon the development of modern electrical apparatus, which visualizes action at a distance. Technique is the means by which the nature of the object is observed, and the result of the observation is no more reliable than the technique used.

The mature mind is affected by the *nature* of the object. It is stimulated to ask questions concerning that which has been observed. Although the mature mind is critical of the techniques used for the exploration of the nature of the object, it has faith that they are capable of revealing something about it. After the method is evaluated the logic of conclusion follows, and the mind states what the technical method has revealed. The next step taken is that of comparison between possible interpretations and an exercise in judgment chooses the most logical. Thus the researcher is or becomes a creative thinker.

Any premedical science in the medical curriculum can be presented in the manner in which a research worker investigates his own peculiar object of interest. Instead of presenting a so-called fact dogmatically the techniques used by a certain investigator can be described and evaluated. It can be shown how in the light of these techniques, his conclusions follow and "fact" is established. On the other hand, if another investigator in the same field has claimed to have established a different or contradictory "fact," his technique can also be described and evaluated, and the rationale of his experiment, discussed. The teacher may be able to show that one of these two has not used reliable methods or has confined his field of observation to, too narrow a radius. As the student listens to this exercise of critical judgment, he becomes familiar with the methods of creative thinking. The impact of this method of the presentation of a subject upon the immature mind creates for months a certain consternation. The more mature individuals among the student group are delighted, for they are beginning to get a glimpse of how to use their own critical faculties. The protest of the former group is difficult to bear. They protest as loudly as a healthy baby who does not get exactly what he wants. And yet before the first year is over, the students who lamented the loudest have become the most critical of didactic and dogmatic teaching. They have begun to mature, for they have discovered the joy which using their own minds upon material presented, is

able to give them; and they resent a subject so presented that memorizing is the only cortical activity they are allowed to use.

Thus in time, (and sometimes in an astonishingly short time) tenacious subservience to the textbook gives way to learning directly by examination of material. When they learn to examine the object for themselves, to see it as it exists under their microscopes, they will become interested in the subject and begin to think with their own cerebral tissue. The reading of original papers becomes a natural part of their preparation, because the awakening of their critical powers has given meaningful expression to their intellectual faculties. These discoveries are intoxicating, for now they have become actors in the subject, beginning to develop by exercise the weighted judgment so necessary for the fulfillment of their later professional activities. But beware! At this stage the exercise of their powers is not limited to the subject. Oh no, they turn their critical ability upon teachers as phenomena to be observed and evaluated.

So presented, Anatomy (histology or neurology) becomes a subject in the process of creation. No one is finished, in spite of the mitotic activity of textbooks. And before the course is over, some of those who were patently young on the first day have looked up at me and said, "All is not known about Histology."

And I have answered, "Indeed not. Histology is not finished, nor is any subject you will study in the next three years. Its future is yours. If all were finished, what would there be left for you to do?"

Our knowledge of the structure, our interpretation of the function of each part of the animal body has a history. It did not spring full blown from the forehead of Jove, nor was it plucked like an apple from the tree of knowledge, nor can it be invented, 15 minutes before a lecture. Rather, it was created in time by men, men who once were young. Some of these men were great in spirit as well as in mind. It is good, I have found, to tell the stories of such men, of their

devotion and of their dedication to their chosen work. If the subject is one which I have seen develop, I tell them what I as a student was taught. If I have known the principal actors in its development I tell their story. Wherever possible I quote the work of students I have taught. I say "These men were once as young as you are. They sat where you are now sitting. They knew no more than you know now." And sometimes I quote my former teacher, who has lived his faith, that "it does not matter who is right; the only important question is, what is true?"

Under the impact of the knowledge of men who preceded them, with the dawning realization that there is something for them to do, their idealism breaks its forged bands, and a future in which they can play a part opens before them.

After 4 or 5 months of this type of teaching they glimpse that they and I are working together. They begin to feel *the need* to understand the processes which characterize living matter. As this need becomes intelligent, voluntarily supported and sustained they create their own internal discipline. Tests and quizzes are unnecessary, for they have come upon the joy of using their own minds in creative thinking. When this type of interest becomes dominant, energy is available and the mastery of a subject is no task. *They have been nourished by the vision of a field of knowledge in the process of creation by the work of men like themselves.* They are no longer hungry, for they have been fed with meat for men.

Each student who reaches this maturity will continue throughout his life to explore, for he has an inward necessity to continue his own education. Such a one will know clearly what he does and does not know. The line will be sharp. And if he has met generosity, he will mete out generosity to his fellow doctors, to his patients and to his friends. From such an inner discipline is born the tolerance required of the profession in its daily impact with human tissue. Such men will be able to look upon themselves as investigators of the attempts made by the human body to function against a background of pathology. They will find few textbook pictures, for the

sick present eternal variations upon the pathological norm. Having broken with the rigidity of youth, they will be able to be tolerant of the variations, presented by the sick or by those who clamor for attention.

Oriented toward the future, with an understanding of the past growth of medicine, knowing that change is the natural state of animal tissue, of society and of man himself, our student will be able to grow in wisdom and in the knowledge which the future holds. He will be strong enough to meet events in society and in his profession in this time of accelerated change.

At this point it is well to stop and remember that the last assay of medical education in this country found that the Departments of Anatomy in almost half of our 4-year medical schools gave no real scientific training to their students as they taught them Anatomy. For the faculties of these schools had no interest in research or research programs. Their teaching was necessarily didactic and dogmatic. Further, there are individual members of faculties of Anatomy (and of other subjects, as well) who never let their methods of research transgress their field of teaching. It is entirely possible that at least a third of the 5,000 who become newly licensed physicians in 1949 have not been scientifically trained in this sense: and of the remaining number how many will use their scientific training in the practice of their profession?

At this time in which we live, the scientist, whether he be a physician or a physicist, is called upon to be a citizen. He needs now as never before a social orientation and an understanding of human interactions. If, in this age of social and economic transition, scientifically trained doctors are to emerge from *all* of our medical schools as intelligent citizen leaders, a fundamental overall change will have to affect *all* medical education.

The research method of teaching is the only method I know which naturally incorporates the concept of growth and change in the presentation of the study of living tissue. The research method of teaching is the only method I know which allows

the twin functions of research and teaching to grow in true bilateral symmetry. This method does not differentiate the teaching of future practitioners of medicine from the teaching of future Anatomists. The teaching of both, assuredly, is dependent for its most perfect realization upon the capacities of the individual student. The teacher of Anatomy in our medical schools must not only provide for the scientific training of future teachers of Anatomy and future practitioners of medicine but also develop personalities capable of adding fresh insight to the accumulated stores of the traditional disciplines of medicine and of creating understanding of new knowledge.

This is a statement of my faith, forged from experience, not only of my years of teaching but also of my years as a student. For, in the Department of Anatomy at the University of Chicago I had two great teachers, one whose faith I have quoted, Dr. Robert R. Bensley, and the other, with whom I have spent many delightful hours, first as a student technician, second as a graduate student and third as a colleague, our President, Dr. George W. Bartelmez. From these two I learned the methods of research I have used, from these two, these methods of teaching. My debt cannot be paid to them. I have tried to pay that debt by investing what I owe in my students.

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MICROSCOPIC STUDIES OF THE TEETH OF A 6-YEAR-OLD-BOY ¹

I. TOOTH ERUPTION

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TEN FIGURES

INTRODUCTION

The purpose of this study is to add to our knowledge concerning the histology of the tooth during eruption. Eruption involves the migration of the developing tooth from its interosseous location to its position of occlusion within the oral cavity. A survey of the literature shows that controversial and inadequate information exists.

This report on a single individual is presented because of the rarity of similar material, and nowhere in the literature do we find a complete investigation of both jaws and all the teeth from one person.

A review of the literature concerning eruption was published by Schour and Massler ('41), and in their evaluation of 7 different theories of eruption they came to the conclusion that vascularity of the periodontal tissue was the most important factor in explaining this process. As a result, the dental sac, the precursor of the periodontal membrane was

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chosen as the basis for particular study because it appears to play a greater role in the eruptive process than has been appreciated previously.

Orban ('28) concluded that the root of the erupting tooth does not grow into the alveolar bone, reasoning that Hertwig's epithelial root sheath acts as a fixed point. Weinmann ('41) used the bony changes of the developing alveolus to subdivide the eruption process into three phases. The first includes the movement of the tooth germ preparatory to eruption, and is characterized by slow appositional bone growth. The second phase coincides largely with the development of the root and shows rapid bone formation. The third exhibits slow bone apposition, and starts at the time when the root is completely formed and lasts throughout the life of the tooth.

Sicher ('42a) seeking the causes for axial movement of continuously growing and erupting teeth of rats, explains that the pulpal growth alone is responsible for their eruption; that is, the pulp grows in a restricted proliferative zone at the basal end just above the unfolding of the epithelial root sheath. This growth is simultaneous with and equal to the growth of the epithelial root sheath at the region of the infolding. This pulpal growth is directed in its effect by a "hammock ligament" which is anchored in the bone above the alveolar fundus and supports the basal end of the tooth. This ligament protects the bone at the fundus of the developing tooth from resorption but allows an outgrowth of the tooth. In a later paper Sicher ('42b) described the same condition in the eruption of teeth of limited growth. However, he adds, "The cushioned hammock ligament correlates root (pulpal) growth and bone (osteogenic tissue) growth to one co-ordinated force of eruption." He found no hammock ligament in the multi-rooted teeth.

MATERIAL AND METHODS

The material used in this study consisted of teeth from the mandible and maxilla of a 6-year-old boy who died from traumatic injuries. Both the mandible and maxilla were fixed

10 days in a formol acetic acid 80% alcohol fixative (5 cm³ formalin; 5 cm³ acetic acid; 90 cm³ 80% alcohol). Ten percent nitric acid in formalin was used as a decalcifying agent, because its penetration is rapid thus preventing excessive action of the acid on the soft tissue. The jaws were cut into blocks, each containing one tooth. The blocks were prepared for sectioning by the paraffin and the paraffin celloidin methods and were cut into sections 10 to 150 μ thick. The thicker sections were used to give a third dimensional effect as demonstrated by Bernick ('48) in his work on the innervation of the human teeth.

The sections were routinely stained with hematoxylin and triosin, Mallory's connective tissue stain, Verhoeff's elastic tissue stain, and Pearson's silver impregnation method.

OBSERVATIONS

In this study of the changes in eruption two structures surrounding the developing permanent tooth were chosen for investigation; the dental sac and the bony alveolar capsule. Using the anatomical stages described by Noyes, Schour, and Noyes ('47) as a basis, the three early stages were confirmed; (1) the preparatory stage, observed in the second permanent molar and the bicuspids; (2) the migratory stage, presented in the lateral incisors; and (3) the emergence of the crown tip, exhibited by the central incisors and the first molars.

The dental sac is composed of the connective tissue or its products surrounding the enamel organ and the dental papilla, all confined within the bony alveolar capsule. This connective tissue was chosen as the basis for particular study because it appears to play a greater role in the eruptive process than has been appreciated previously. In the preparatory stage the dental sac differentiates into three distinct layers: (1) outer; (2) intermediate, and (3) inner layers (fig. 1).

The outer or periosteal layer consists of a thin layer of dense collagenous fibers staining prominently with the blue of Mallory's connective tissue stain, and deep red with Van

Giesen's stain. Through it the blood vessels, lymph vessels, and nerves come from the adjacent bone.

The intermediate layer, consisting of loose connective tissue, shows a differentiating staining reaction. With Mallory's stain the intercellular ground substance appears to be viscid, or gel-like, in its nature and stains red. Interposed within the ground substance are fluid spaces which do not stain, indicating an edematous condition. The stroma is composed of reticular fibers which stain blue with Mallory's stain and impregnate with silver (fig. 2). In addition a few elastic fibers are seen scattered throughout this stroma. In the region of the alveolar fundus of the developing tooth within the circle of Hertwig's epithelial root sheath, this tissue adheres closely to that of the dental papilla.

The inner layer, except for being wider, is similar to the outer periosteal layer in composition and staining reaction. It is in direct apposition to the reduced enamel epithelium and adheres closely to Hertwig's epithelial root sheath at the fundic region of the developing tooth. Blood and lymph vessels pass from the alveolar bone through the outer periosteal and intermediate layers terminating in the inner layer, giving off branches throughout their course. Capillary loops from the inner layer enter the reduced enamel epithelium.

As the migratory phase appears, changes occur in the dental sac. In the coronal third on the occlusal surface the dental sac becomes transformed into a disorganized wide layer. Its structure is a diffuse connective tissue, staining blue with Mallory's stain. Its fibers are dense collagenous elements which run in all directions (fig. 3). From the coronal third to the fundic area, the dental sac still exhibits a differentiation into three layers. The outer periosteal layer has become thicker and denser, containing osteoblasts and osteoclasts which are adjacent to the bone. At this stage of development there is no indication of the formation of Sharpey's fibers. The intermediate layer has become narrowed and is arranged as diffuse tissue between which are found cleft-like spaces containing a small amount of tissue fluid. At the

fundic portion this layer fans out in a network of delicate precollagenous bundles between which are found fluid spaces (fig. 4). The inner layer is similar in composition to the outer fibers except that differentiation into cemental fibers has already begun (fig. 5). In the fundic portion of the root this inner layer lies in intimate association with all of Hertwig's epithelial root sheath and extends a short distance beyond it, blending with the connective tissue of the dental papilla and intermediate layer.

The central incisors are in the stage of emergence of the crown tip. In the region of the upper two-thirds of the root the fibers of the dental sac have differentiated into irregular periodontal fibers. Sharpey's fibers are here observed in the newly formed alveolar bone and cementum. At the fundic region these fibers again divide into the previously described three distinct layers i.e., the inner layer closely adhering to Hertwig's epithelial root sheath; the intermediate layer which is still the widest and consists of a network of precollagenous elements rich in blood vessels, lymph vessels, and nerves; the outer periosteal fibers which are adjacent to the developing alveolar bone (fig. 6).

The crypt of the permanent tooth is surrounded by a bony capsule which shows considerable bone formation and bone absorption during the above mentioned stages of eruption. In this description the bony covering of the crypt will be divided roughly into three areas: (1) occlusal; (2) buccal or lingual, and (3) fundic. Each area is divided into an inner and outer portion in relation to the dental sac.

In the preparatory stage the occlusal portion of the capsule on its outer surface shows an active bone formation while on the inner there is a bone absorption (fig. 7). The lateral portion above the height of contour, which is the greatest diameter of the tooth in a horizontal plane, osteoclasts are present on the dental side while on the outer surface there is osteoblastic deposition (fig. 8). This appears to be an expansion process, making room for the eruption of the tooth. Below the height of contour there is bone formation on both

sides indicating a strengthening process. At the fundic area bone formation is also found on both sides (fig. 10). At this stage of eruption trabeculation of the bone first appears.

In the migratory stage the occlusal portion of the bone is completely absorbed. The primary tooth is present, and there is no bone between it and the dental sac of the erupting permanent tooth. Laterally above the height of contour in the lower lateral incisor, there is bone absorption on both sides and even extending into the area surrounding the primary tooth. In the upper lateral incisor bone absorption is found on the labial side while bone formation occurs on the palatal or lingual which is away from the bony crypt. Below the height of contour bone formation predominates with a thickening of the alveolar bone on the palatal side. There is further bone formation in the fundic area which exhibits trabeculation.

In the stage of emergence of the tooth, represented by the incisors, the changes in the bony capsule are restricted to the lateral and fundic areas. Laterally above the height of contour the alveolar bone has narrowed considerably and continued bone absorption is observed. Below this area there is still bone formation with apparent thickening of the alveolar bone. No further difference is found in the fundic area other than that previously described, except for an increased bone growth.

DISCUSSION

Eruption of the tooth is not a growth phenomenon, but the movement of the tooth through the different types of tissue within the jaw. Opinions differ in regard to the mode and forces involved in the migratory process. Some workers have tried to explain the migration in terms of dental tissue growth; others have attempted to demonstrate the process by absorption of the alveolar bone; and finally a number of investigators have tried to show that changes in the vascularity of the periapical tissue plays an important role in the eruptive process.

Descriptions of the changes in the dental sac and bony capsule surrounding the crypt of the erupting permanent tooth are at best inconclusive. Many authors have indicated that changes in this tissue may be a contributory factor in tooth eruption. Sicher ('42) described, in rat and man, the dental sac differentiating into two layers at the region of the fundus. The first closely attached to the bone and consisting of loose connective tissue, whereas the second lying adjacent to the growing end of the tooth, consists of a network of rather thick fibers and contains a large amount of fluid in the tissue spaces between the fibers. Strong strands of fibers from the periodontal area at the side of the root curve as a strong ligament around the edge of the root and then divide into a network forming spaces that are filled with fluids. This is designated by Sicher as "hammock ligament."

Sicher's contention that the hammock ligament is an aid in the eruptive process is quite accurate as far as it goes. His limiting of the hammock ligament to the fundic area and to two layers is based on the fact that he only observed teeth in the later stages of eruption when there is no bone between the permanent tooth and the primary tooth.

In this study it was found that the dental sac undergoes certain changes in structure and affinity for various stains as the tooth migrates in eruption. A study of all stages of eruption indicate that there are three distinct layers of the dental sac in the preparatory stage. The inner dental and outer periosteal layers are of dense connective tissue only varying in thickness. On the other hand the intermediate layer is of loose connective tissue made up of reticular fibers. Its intercellular ground substance shows a differential staining reaction with any of the connective tissue stains, i.e. it stains red with Mallory's. Interposed throughout this layer are tissue spaces indicating edema.

In the subsequent stages of eruption, the outer periosteal and inner dental fibers become orientated into attachment fibers. The cemental fibers precede the alveolar group in development. At the fundic portion of the root the inner fibers

still adhere closely to Hertwig's epithelial root sheath, extending, and ultimately blending with the connective tissue of the pulp. This relationship is noticeable in thick sections.

The stroma of the intermediate layer become collagenous in nature and blends in with those of the inner and outer layers above the coronal third of the tooth. Below this area and around the fundus of the root, the intermediate layer remains loose and edematous. During these periods there is no bone separating the primary tooth from its successor. As the tooth enters its eruptive stage, fibers are clearly differentiated, thereby allowing themselves to become the periodontal fibers of the erupting tooth.

Although the controversy in the literature concerns itself with the mode and forces involved in the migratory process, little is known about the histological changes of the bony capsule surrounding the permanent tooth. It is well known that in the movement of the tooth, bone absorption must occur enabling the tooth to move through the bone. In general, this is what one finds with the human tooth. In the early stages of eruption bone absorption is restricted to the area above the height of contour. The bone is not completely destroyed, because while there is bone destruction on the dental side of the bony capsule, bone formation prevails on the outer side indicating a supportive process. Below the height of contour there is bone formation on both sides of the bone indicating a strengthening process. As the tooth erupts further this relationship continues until occlusion is complete. It is probable, therefore, that apposition of bone in certain restricted areas is one of the primary moving agents.

It may be concluded that differences in the descriptions of the dental and osseous structures involved in eruption have been due to incomplete studies of all the teeth at any one age.

SUMMARY

1. In the jaws of a 6-year-old boy the changes of the dental sac and bony crypt of the developing tooth were studied under differential stains during the first three stages of eruption.

2. In the preparatory stage of eruption the dental sac was found to consist of three layers: (1) the inner dental layer is wide and of dense connective tissue; (2) the intermediate layer is of loose connective tissue whose stroma is made up of reticular fibers among which are fluid spaces indicating an edematous condition; (3) the outer periosteal layer is similar to the inner in consistency only differing in thickness.

3. In the succeeding two stages of eruption, migratory and pre-clinical, the layers of the dental sac become transformed into disorganized attachment fibers except at the fundic portion of the developing tooth where they are still recognized as three separate layers.

4. As the tooth further erupts, these early attachment fibers gradually differentiate into the periodontal membrane; the cemental fibers precede the alveolar group in development.

5. In all eruptive stages studied bone absorption appears to be restricted to the area above the height of contour of the erupting tooth within its bony crypt. Below this area on both sides of the bony capsule, bone formation occurs indicating a strengthening process.

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PLATE 1

EXPLANATION OF FIGURES

1 Lower first bicuspid. Dental sac consisting of three layers. E.P., enamel of permanent tooth; D.S., dental sac; B.P., bony capsule; 1, inner dental layer; 2, intermediate layer; 3, outer periosteal layer. Mallory's connective tissue stain. $\times 50$.

2 Lower first bicuspid. (Intermediate layer.) Note reticular fibers and the fluid spaces. F.S., fluid spaces; R.F., reticular fibers. Pearson's silver gelatin impregnation. $\times 775$.

3 Upper primary and permanent lateral incisors. Note the lack of bone between the two teeth. E.P., enamel of permanent tooth; u.e.e., united enamel epithelium; D.S., dental sac; P., pulp of primary tooth; D., dentin of primary tooth. Pearson's silver gelatin impregnation. $\times 30$.

4 Fundic area of lower permanent incisor during the migratory stage. Note the fluid spaces. D.P., dental papilla; B.V., blood vessels; F.S., fluid spaces; Ob., osteoblasts; B., bone. Hem. and Tr. stain. $\times 130$.

5 Lower permanent lateral incisor. Note the differentiation of cemental fibers. D., dentin; C., cementum; C.F., cemental fibers; P.M., periodontal membrane; B., alveolar bone. Pearson's silver gelatin impregnation. $\times 300$.

6 Upper permanent central incisor in preclinical eruption. Fundic area of the tooth. Note the three layers of the dental sac. D.P., dental papilla; Od., odontoblasts; D., dentin; H.S., Hertwig's epithelial root sheath; B., bone; 1, inner dental layer; 2, intermediate layer; 3, outer periosteal layer. Pearson's silver gelatin impregnation. $\times 130$.

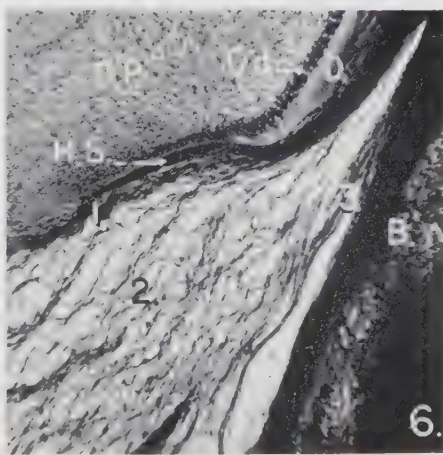
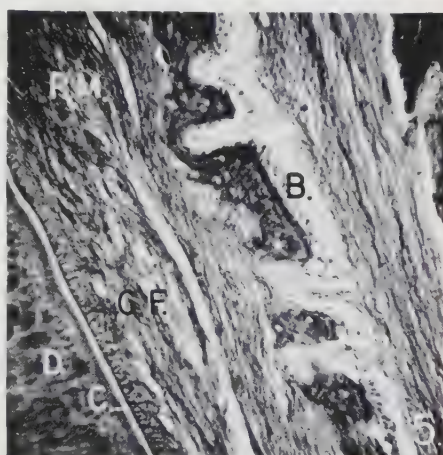
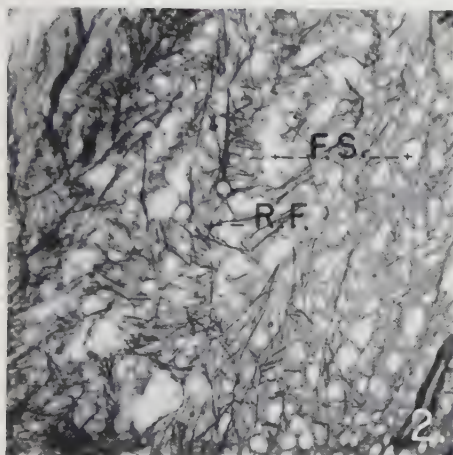
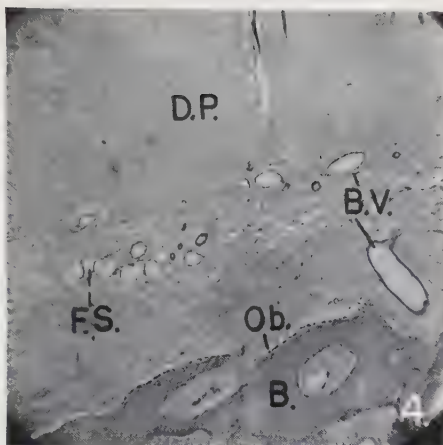


PLATE 2

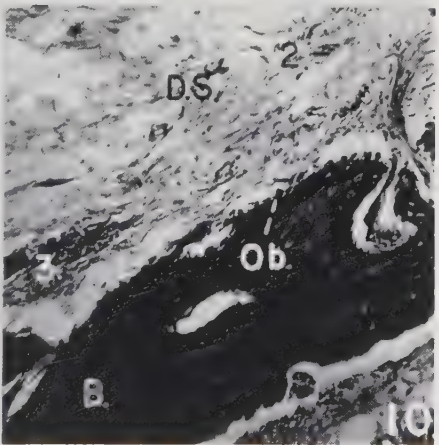
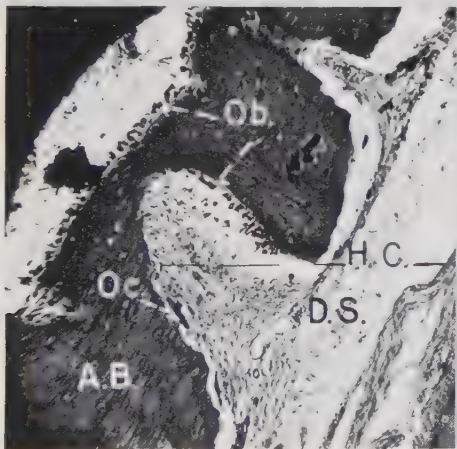
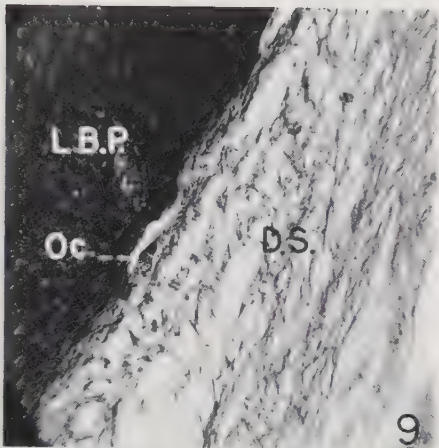
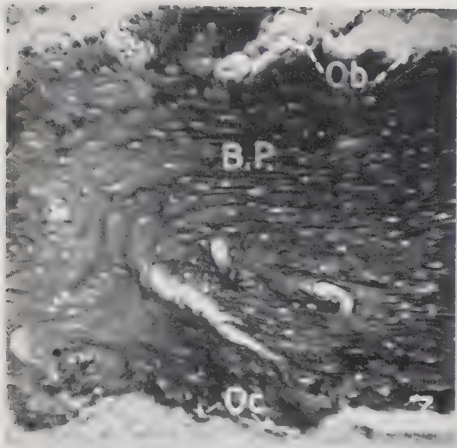
EXPLANATION OF FIGURES

7 The occlusal plate of the bony capsule of an upper bicuspid. Note osteoclasts on the inner portion and the osteoblasts on the outer portion. Oc., osteoclasts; Ob., osteoblasts; B.P., bony capsule. Mallory's connective tissue stain. $\times 300$.

8 Upper permanent cuspid at the height of contour. Note osteoclasts above the height of contour and osteoblasts below. H.C., height of contour; Ob., osteoblasts; Oc., osteoclasts; A.B., alveolar bone; D.S., dental sac. Hem. and Tr. stain. $\times 130$.

9 Upper permanent cuspid. Note osteoclasts laterally above the height of contour. L.B.P., lateral bony plate; Oc., osteoclasts; D.S., dental sac. Pearson's silver gelatin impregnation. $\times 300$.

10 Upper first bicuspid. Note active bone formation at the fundic portion of the bone. D.S., dental sac; Ob., osteoblasts; B., bone; 2, intermediate layer; 3, outer periosteal layer. Mallory's connective tissue stain. $\times 130$.



MICROSCOPIC STUDIES OF THE TEETH OF A 6-YEAR-OLD-BOY¹

II. TOOTH ABSORPTION

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FIFTEEN FIGURES

INTRODUCTION

This investigation involves a detailed histological study of the shedding of the primary teeth of a healthy 6-year-old boy who died from traumatic injuries. The material studied, is from the same specimen as that reported in part I of these studies by Bernick et al. ('49).

Linderer (1851) was the first to give us a detailed description of the changes that occur during the process of shedding. He stated that, "roots of the temporary tooth are resorbed by the follicle of the permanent tooth." J. Tomes (1859) was the first to describe the osteoclastic resorption of the roots of the deciduous teeth. Charles Tomes (1876) in a review of the contemporary knowledge concerning the elimination of the deciduous teeth emphasized the following points: (1) "absorption is not restricted to one side of the tooth but occurs in many places at once. (2) the process of absorption once commenced does not necessarily proceed without inter-

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mission but may give place for a time to actual deposition of osseous tissue on the eroded surface. (3) the cementum is usually attacked first but eventually the dentine and even enamel come to be scooped out and removed by an extension of the process. That part of the dentine which surrounds the pulp appears to have more power of resistance than any other part of the tooth and thus persists for a time as a hollow column, i.e., cuspid resorption." Pommer (1883) found that in a case of simultaneous resorption of calcified and uncalcified bone, the uncalcified tissue seems to have more power of resistance against absorption. Oppenheim ('22) found that during the eruptive phase both the alveolar bone and deciduous tooth are absorbed to a larger extent than the actual movement of the permanent tooth would necessitate and this excess absorption is made up for by a reparative new formation of bone and cementum in the period of rest. Gottlieb ('21) and Kronfeld ('27) have shown that during resorption of deciduous teeth the uncalcified predentin remains intact whereas the surrounding calcified dentin is absorbed. Hope-well-Smith ('30) came to the conclusion that in the cat the pulp tissue itself takes part in the resorption of the dentin of the deciduous tooth. He found osteoclasts on the inner wall of the pulp chamber hollowing out the tooth from within. Marshall ('28) working on animals gained the impression that resorption of the deciduous teeth occurs as a rule from within outwards. Kronfeld ('32) came to the conclusion that the roots of the deciduous teeth are absorbed by the connective tissue between the permanent and deciduous teeth and that the absorptive process begins on the side of the root facing the crown of the permanent tooth although sometimes it might occur on the opposite side. That part of the dentin which immediately surrounds the pulp has greater resistance against absorption than any other part of the tooth. He further stresses that in man, the pulp does not participate in the process of absorption whereas in the cat and dog it does.

MATERIAL AND METHODS

The material used in this study consisted of teeth from the mandible and maxilla of a 6-year-old boy who died from traumatic injuries received in an automobile accident. Both the mandible and maxilla were fixed in a formol acetic acid, alcohol fixative (formalin 5 cm³, acetic acid 5 cm³, 80% alcohol 90 cm³) for over a week. Ten percent nitric acid in formalin was used as a decalcifying agent because its penetration was rapid, thus preventing excessive action of the acid on the soft tissue. The jaws were cut into blocks each containing one tooth. Paraffin and paraffin celloidin sections of the blocks were prepared after decalcification. Sections were from 10 to 150 μ thick. The thicker sections were used to give a third dimensional effect.

The sections were routinely stained with either hematoxylin and triosin, Mallory's connective tissue stain, Verhoeff's elastic tissue stain, or Pearson's silver gelatin impregnation method.

OBSERVATIONS

The shedding of the primary teeth is at least partly the result of the progressive action of osteoclasts or their agents on tooth structures. The pressure exerted by the erupting permanent tooth may be a factor causing a proliferation of the osteoclasts. It is commonly believed that these cells produce a lytic substance which dissolves the bone and tooth structure, but there is no direct evidence to support this belief. Osteoclasts are usually seen where bone and tooth substance are being dissolved and are frequently found in deep recesses both in bone and tooth structure. When absorption of the bone and tooth ceases in a particular location, the osteoclasts disappear; most of them apparently becoming osteoblasts or reticular cells depending on the type of stimulus. In this presentation developmental absorption is limited to that absorption caused by the pressure exerted by the erupting teeth, whereas any other shallow root absorption is called traumatic absorption.

Lateral incisors. Sections of the upper and lower lateral incisors show that these teeth are in the last stages of developmental absorption because the roots are almost completely destroyed. The crown is only superficially attached to the gingiva. There is no bone between the primary teeth and their permanent successors in any area (fig. 1). The epithelial attachment has proliferated along the remaining portion of the root and has nearly reached the absorbing area (fig. 2). This proliferation appears to progress much faster on the lingual surface of the interproximal space due to the fact that the permanent incisor teeth erupt lingually. As a result of the epithelial proliferation, the attachment fibers appeared to be severed. Immediately under the epithelium lymphocytic infiltration takes place (fig. 3). The pulp of the primary teeth has been invaded by granulation tissue which includes the osteoclasts. This tissue arises from the connective tissue of the dental sac surrounding the permanent tooth. The osteoclasts align themselves along the pulpo-dentinal junction toward the cementum (fig. 4). This pulpo-dentinal absorption is not uniform in its progression. In figure 5 osteoclasts are seen in recesses in the predentin at different areas in the crown portion of the pulp. Soon these individual areas coalesce to form one large absorbed site (fig. 4). Once the pulp has been invaded by osteoclasts the absorption proceeds in all directions by hollowing out the predentin, dentin, and lastly the cementum. The hollowed portion is replaced by granulation tissue, and repair of the eroded area by bone-like material is possible (fig. 6).

Individual areas of absorption are seen on the labial side of the cementum, but no osteoclasts are present, and fibroblasts and osteoblasts are observed (fig. 7). After a Mallory's connective tissue stain or a silver impregnation technic, newly formed attachment fibers are also noted in the same field. This absorbed region is due to traumatic stresses. The remaining alveolar bone is undergoing extensive absorption.

Cuspids. The cuspid teeth show the beginning stages of developmental absorption. In both the lower and upper cuspids

the epithelial attachment has just begun to proliferate rootward. Inflammatory cells are seen directly underneath the epithelium. Serial sections of the lower cuspid tooth show absorption at the lingual surface in the apical third of the tooth. However, the absorption is restricted to an area where no bone separates the primary tooth from the crypt of the permanent crown. As long as bone separates the teeth, no developmental absorption occurs. Figures 8, 9, 10, and 11 are successive sections of a lower cuspid showing the progression of absorption. In figure 8 there is extensive bone between the two teeth. In the succeeding sections, absorption is demonstrated only when the bone is destroyed. It is to be noted that the pulp at this time is still intact. On the other hand, traumatic absorption occurring with repair is evident on the labial surface of the roots as indicated by shallow erosions on the surface. Repair of these surfaces is by a substance which appears to be identical with newly formed bone. Since chemical studies have not been undertaken, this substance will be referred to as a "bone-like" material. Here an area of newly formed bone-like substance is shown along side the dentin (fig. 12). Simultaneously with tooth absorption, there is bone absorption. During the period of rest, repair of bone and tooth occurs. In the upper and lower cuspids, newly formed bone is being deposited. With Mallory's connective tissue stain and silver impregnation, argyrophilic fibers are observed forming the matrix of the newly formed bone and osteoblasts are seen lined up next to the bone (fig. 13).

The absorbed area on the upper cuspid is found on the lingual surface at the apical third. Here as in the lower cuspid there is no bone between the primary and permanent tooth in the region of the absorption of the root of the primary tooth (fig. 14). The degree of absorption has reached the point where only a sliver of dentin separates the connective tissue of the dental sac and the pulp. It is evident that repair has occurred on this surface, for newly formed bone-like material has been deposited on the eroded surface (fig. 13).

First primary molar. The first primary molar shows intermediate stages of absorption. The epithelium is proliferating rootward just separating the periodontal fibers. Again chronic inflammatory cells are seen underneath the proliferating epithelium.

The developmental absorption in this case is restricted to the surface of the roots next to the inter-radicular septum. One root has been completely absorbed, and granulation tissue has just invaded the pulp, and plates of newly formed bone are present underneath the invading granulation tissue. Again there is no bone between the absorbed area and the connective tissue of the dental sac. Traumatic absorption and repair by a bone-like material is evident at the bifurcation (fig. 15). In one of the upper first molars the repair of the roots has reached a degree that ankylosis by bone tissue is demonstrated.

Second primary molar. The second molars show no developmental absorption at this time but do show evidence of traumatic absorption.

DISCUSSION

It is agreed that absorption of the primary teeth is the result of pressure exerted by the erupting permanent teeth. This absorption is characterized by the presence of osteoclasts in bone and tooth structure. However, there is a lack of agreement regarding the mode of absorption. Some workers have restricted tooth absorption to the cementum only while others believe that pulpal action does occur. It is agreed that absorption of the primary teeth is the result of the action of osteoclasts on both bone and tooth structure. However, there is a lack of agreement regarding the mode and action of these osteoclasts. Some workers have restricted their activity to the cementum of the tooth while others believe that they act also in the pulp.

To discuss properly the absorption of primary teeth it is necessary to differentiate between traumatic and developmental absorption. The experiments of Gottlieb ('21), Orban ('28), and Oppenheim ('22) on the influence of functional

stress upon the teeth of dogs and man have shown that occlusal trauma is able to cause root absorption, which is usually shallow in nature and shows microscopic repair as the trauma subsides. These are insignificant in comparison with the developmental absorption. In the mixed dentitional period especially at the age of 6 years, it is well known that masticatory forces increase due to the growth of the masticatory muscles which increases the stresses and acts as a traumatic force upon the teeth. The forces are, consequently, transmitted to the alveolar bone and tooth causing, first, necrosis of the periodontal membrane and later, absorption of the tooth structures.

On the other hand, it is assumed that developmental absorption is the result of the pressure exerted by migration of the permanent teeth through the overlying bone. Because of the position of the permanent tooth, the absorption of the anterior primary teeth starts at the lingual surface in the apical one-third of the root, while in most cases the absorption of the primary molars begins on the surface next to the inter-radicular septum.

Developmental absorption begins only when the bony plate separating the primary tooth and its permanent successor has been absorbed. As long as bone separates the two teeth, no developmental absorption occurs. Pressure from the migration of the permanent tooth is first exerted on the bony plate. At this stage the osteoclastic-osteoblastic action appears to be in a neutral state. As the absorption continues from within, bone is deposited on the outside, and when the osteoclastic activity predominates, the bony plate decreases in thickness at the expense of the outer phase until such time that a break through occurs allowing this pressure to be exerted against the supportive tissue of the primary tooth, ultimately resulting in the process of developmental absorption.

Although descriptions in the literature are concerned chiefly with generalized absorption of the root, little is known about the progression of developmental absorption. As soon as the bony plate is broken through, absorption begins on the ce-

mental side. The connective tissue of the dental sac and periodontal membrane proliferate to form a granulation tissue. From this granulation tissue osteoclasts arise which act on the cementum, dentin, and finally invade the pulp.

Participation of the pulp in the process of shedding is another important question. Several authors (Tomes, 1876, and Marshall, '28) in dealing with this subject state that the pulp of the primary tooth forms osteoclasts which hollow the crown by absorbing the dentin from the inside of the tooth. These publications were as a rule illustrated with specimens from animal jaws especially dogs and cats. Kronfeld ('32), on the other hand, believes that this does not occur in the human primary tooth. In the material studied, granulation tissue invades the pulp, and the fibroblasts present appear to give rise to the osteoclasts. Once the pulp has been invaded, osteoclasts are observed at areas along the pulpodentinal junction eroding the predentin and dentin. These isolated sites soon coalesce to form one large absorbed region. There is always transitional change between the granulation tissue and the remaining normal pulp. The absorption proceeds in all directions towards the cementum; this tissue being the last to be absorbed after the invasion of the pulp.

It is accepted that the epithelial attachment of the primary teeth grows downward during the absorptive process. This is observed in the present study. In the earliest stage of absorption, the gingival epithelial attachment begins to migrate rootward apparently severing the cemental fiber thereby eliminating the periodontal attachment. As a result, there is an increase in the clinical crown of the tooth. This proceeds until the epithelium has reached the apex of the absorbing tooth; aiding in its shedding. Directly underneath the thickened epithelium are to be found chronic inflammatory cells. This chronic reaction is probably due to the proliferation of the epithelium and increased masticatory forces.

The process of shedding is not necessarily continuous. Periods of great osteoclastic activity alternate with periods of rest. During the rest periods not only does absorption cease,

but repair may actually occur by apposition of bone-like material upon the absorbed area whether it is cementum or dentin. At the same time, newly formed bone is laid down. The rest periods are not uniform as is shown in the material studied. It is not unusual to find one portion of the root under rest from traumatic absorption while another area will be under active developmental absorption.

The apposition of the bone-like material has been designated by many authors as secondary cementum. However, with various stains one may not histologically differentiate the bone-like material from newly formed adjacent bone. With Mallory's connective tissue stain and the silver impregnation method, all of the constituents of newly formed bone are found in the repairing material that is deposited next to the cementum or dentin. Previous workers have differentiated bone from secondary cementum by the lack of Haversian systems in the cementum. Yet, it is now well known that the Haversian systems are not present in the newly formed bone. In the present study of areas undergoing repair, cells are found imbedded in an argyophilic matrix which at this stage cannot be differentiated from osteoblasts. In one extensive area of repair, an early Haversian system is being laid down complete with blood vessel, lacunae, and concentric lamellae. One should consider the possibility of these cells being osteocytes which have been enveloped by the further formation of primary cementum.

Apposition of bone-like material is not restricted to the absorbed areas of the cemental side. Repair of absorbed areas by the bone-like material is also found in the pulp. As long as granulation tissue is present, potential osteoblasts are present which may give rise to repair on the pulpal side.

Ankylosis of the absorbed surfaces of the primary teeth with the adjacent alveolar bone does occur during the reparative process. If one assumes that the repair material is like bone, it is not surprising that in some cases an excess of bone

formation may cause an ankylosis of the tooth with the surrounding bone such as suggested by the so-called submerged primary molar.

SUMMARY

1. In the jaws of a 6-year-old boy the process of tooth absorption was studied by using various differential stains.

2. The differences between traumatic and developmental absorption are discussed. Developmental absorption was found to begin only when the bony plate separating the primary tooth and its permanent successor was absorbed. As soon as the bony plate was destroyed, the absorption then involved the cementum, dentin, and finally the pulp.

3. Once the pulp has been invaded, the process of absorption then follows in a pulpal cemental direction; the cementum being the last tooth structure to be lost.

4. In the various stages of absorption of the primary teeth, the epithelial attachment proliferates along the root surface towards the absorbed areas, apparently severing the periodontal membrane fibers.

5. The process of absorption is not necessarily continuous. Periods of great osteoclastic activity alternate with periods of relative rest. During the rest period, repair by apposition of a bone-like material on both alveolar bone and the eroded tooth structure occur.

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PLATE 1

EXPLANATION OF FIGURES

1 Upper primary (deciduous) lateral incisor. Note the lack of bone between the primary tooth and its permanent successor. E.P., enamel of permanent tooth; G.T., granulation tissue; P., pulp of primary tooth; D., dentin; A.a., absorbed area. Pearson's silver gelatin impregnation. $\times 30$.

2 Upper primary lateral incisor. Note the proliferation of epithelial attachment and the severing of the periodontal membrane fibers. D., dentin; C., cementum; Oc., osteoclasts; E.A., epithelial attachment; P.M.F., periodontal membrane fibers; G.T., granulation tissue. Pearson's silver gelatin impregnation. $\times 300$.

3 Lower primary lateral incisor. Note chronic inflammation underneath the epithelium. Ep., gingival epithelium; E., enamel; E.A., epithelial attachment. Verhoeff's elastic tissue stain. $\times 130$.

4 Lower primary lateral incisor. Note absorption on the pulpal side of dentin. D., dentin; C., cementum; P.M., periodontal membrane; Oc., osteoclasts. Pearson's silver gelatin impregnation. $\times 130$.

5 Upper primary lateral incisor. Note osteoclastic action of isolated areas at predentin and dentin. D., dentin; Oc., osteoclasts; Pd., predentin; P., pulp. Pearson's silver gelatin impregnation. $\times 130$.

6 Upper primary lateral incisor. Note pulpal repair by bone-like material. B.L.M., bone-like material; D., dentin; P., pulp. Hem. and Tr. stain. $\times 300$.

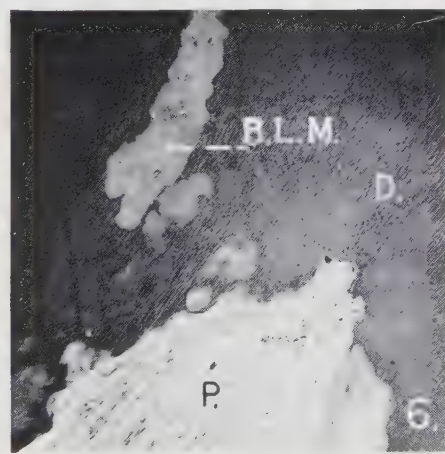
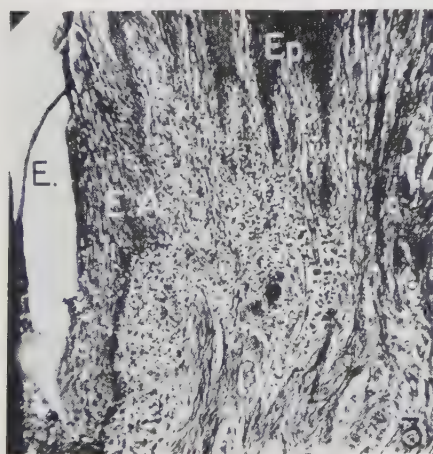
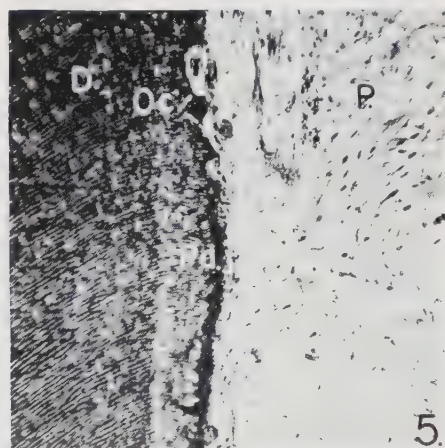
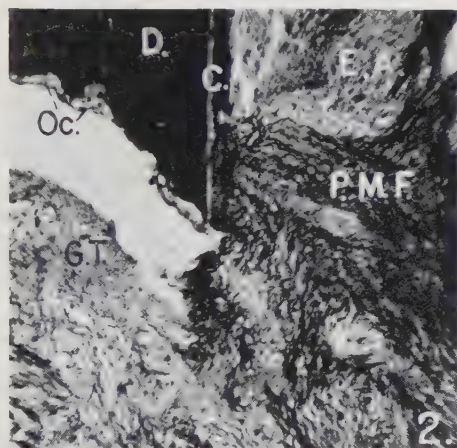
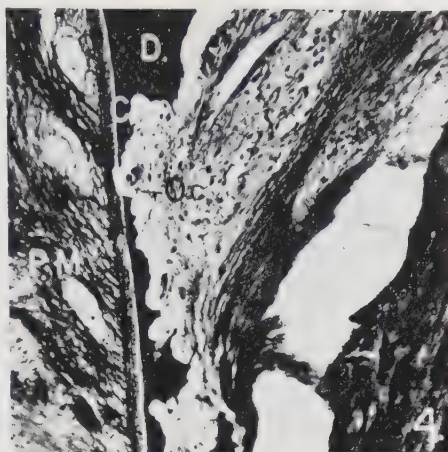


PLATE 2

EXPLANATION OF FIGURES

7 Upper primary lateral incisor. Note traumatic absorption of cementum and dentin. D., dentin; C., cementum; P.M., periodontal membrane; T.A., traumatic absorption. Mallory's connective tissue stain. $\times 130$.

8 Lower primary cuspid. Note bony plate between the root of the primary tooth and the crown of the permanent successor. P.R., root of primary tooth; B.P., bony plate; P.M., periodontal membrane. Hem. and Tr. stain. $\times 30$.

9 Lower primary cuspid (same tooth as fig. 8). Note the beginning breakdown of bony plate. P.R., root of primary tooth; D., dentin; T.A., traumatic absorption; B., bone; D.S., dental sac; E.P., enamel of permanent tooth. Hem. and Tr. stain. $\times 30$.

10 Lower primary cuspid (same tooth as fig. 8). Note further loss of bony plate and the beginning of developmental absorption of primary tooth. P.R., root of primary tooth; D., dentin; B., bone; P.M., periodontal membrane; D.S., dental sac; E.P., enamel of permanent crown. Hem. and Tr. stain. $\times 30$.

11 Lower primary cuspid (same tooth as fig. 8). Further loss of bony plate and absorption of the dentin. P.R., root of the primary tooth; D., dentin; B., bone; D.S., dental sac; E.P., enamel of permanent tooth. Hem. and Tr. stain. $\times 30$.

12 Lower primary cuspid. Note the beginning formation of a Haversian system in repaired dentin. D., dentin; C., cementum; A.B., alveolar bone; H.S., Haversian system; P.M., periodontal membrane. Hem. and Tr. stain. $\times 130$.

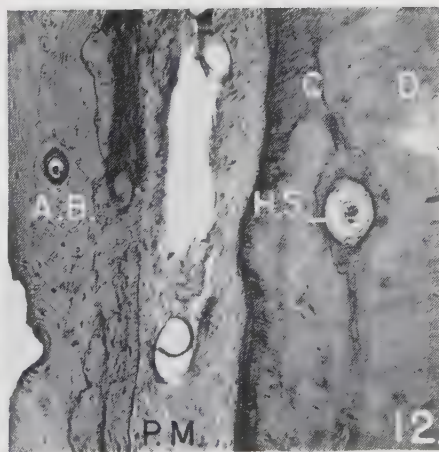
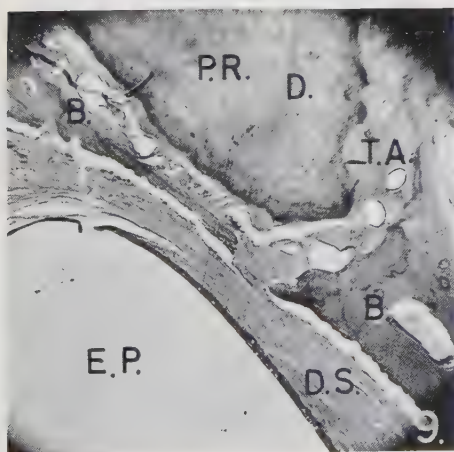
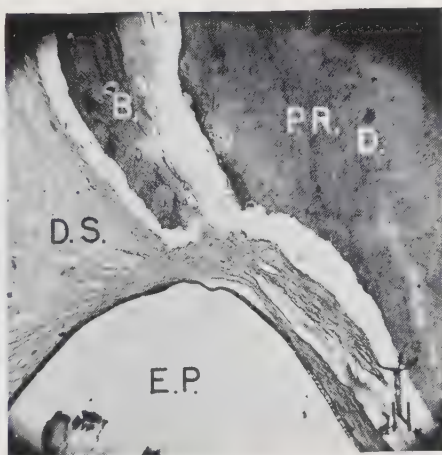
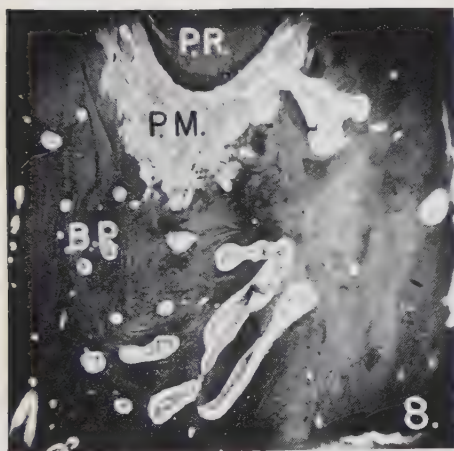
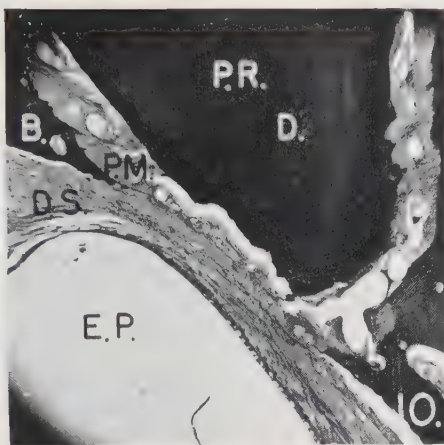
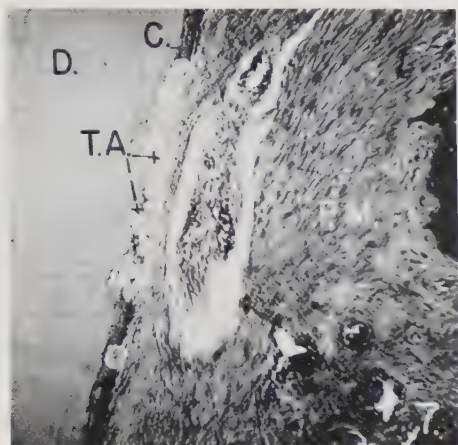


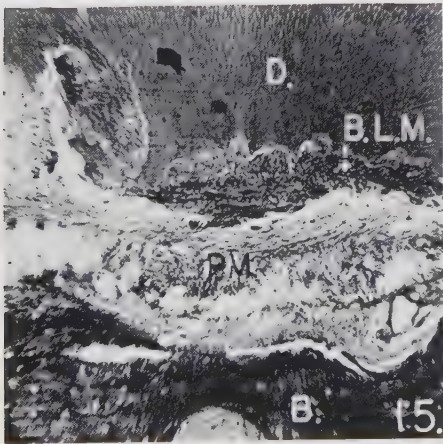
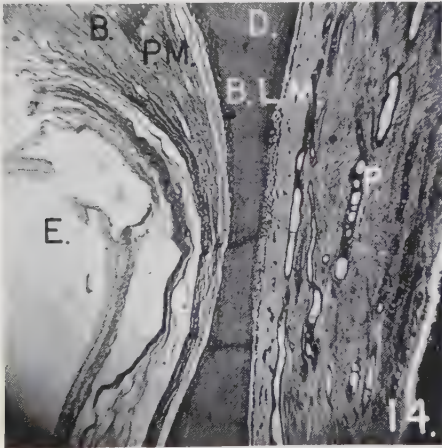
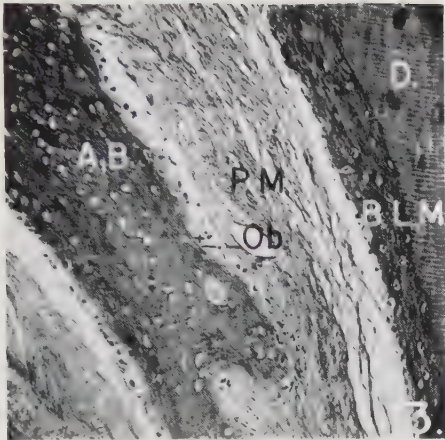
PLATE 3

EXPLANATION OF FIGURES

13 Upper primary cuspid. Note similarity between newly formed alveolar bone and the bone-like material adjacent to the dentin. D., dentin; A.B., alveolar bone; P.M., periodontal membrane; B.L.M., bone-like material; Ob., osteoblasts. Mallory's connective tissue stain. $\times 300$.

14 Upper primary cuspid. Note area of absorption of the dentin and beginning repair by the bone-like material. B., bone, P.M., periodontal membrane; D., dentin; B.L.M., bone-like material; P., pulp; E., enamel of permanent tooth. Mallory's connective tissue stain. $\times 30$.

15 Upper primary second molar. Note area of repair by the bone-like material in a region of traumatic absorption. D., dentin; B.L.M., bone-like material; P.M., periodontal membrane; B., bone; Mallory's connective tissue stain. $\times 130$.



NEW METHOD FOR MICROSECTIONING

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THREE FIGURES

This paper describes an inexpensive, practical method of preparing very thin sections of biological tissue for study with either the light or electron microscope. In the new procedure, *n*-butyl methacrylate is polymerized around the specimen to produce an optically clear embedding medium having highly desirable cutting properties. A smooth, continuous advance of the specimen toward the knife of a slightly modified conventional microtome is then obtained from the thermal expansion of a metal specimen holder. Thus, sections of tissue having uniform thickness, large area, and integrity of structure may be cut one at a time.

An important feature of the method is an easily made, inexpensive device which holds the embedded specimen and at the same time advances it gradually and uniformly toward the knife of the microtome. This device may be employed without modifying the conventional microtome other than to disengage the mechanism normally used to advance the specimen. It is essentially a brass block containing a hole threaded at one end to receive a standard 3/8-inch brass pipe plug. A cavity drilled into the face of the plug provides a seat for the embedded specimen (fig. 1 B). Behind the plug is a needle valve, which admits compressed carbon dioxide gas to the interior of the block. As the gas undergoes a large increase in volume, it cools and contracts the assembly. Stopping or reducing the flow of carbon dioxide allows the apparatus to approach room temperature, and thus the thermal expansion

provides continuous advance of the embedded tissue toward the cutting edge.

In the procedure that was developed, the tissues are fixed and then dehydrated in an ethyl alcohol series by the usual cytological techniques. From absolute alcohol they are transferred to a solution containing equal parts of absolute alcohol and monomeric *n*-butyl methacrylate. After about one hour in the alcohol-monomer mixture, the tissue is put in the monomer

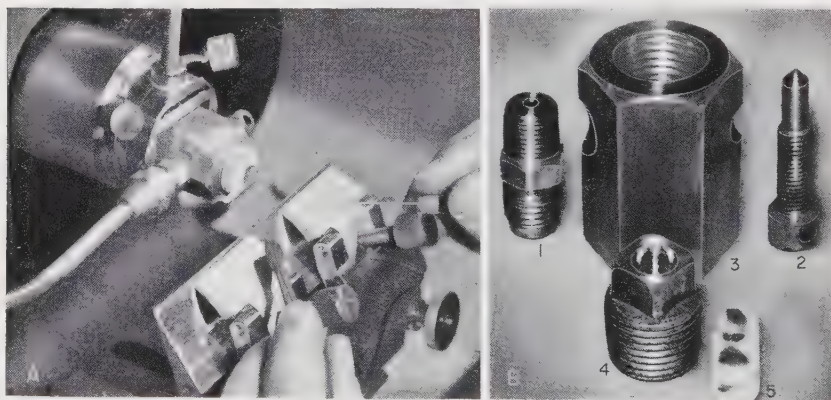


Figure 1

(A) Assembled thermal expansion microtome. A section is being cut with the apparatus clamped in the jaws of a commercial microtome. Compressed CO_2 gas escaping into the expansion chamber cools and contracts the specimen holder. Stopping or reducing the flow of CO_2 allows the apparatus to approach room temperature and thus advances the embedded specimen toward the knife.

(B) Disassembled thermal expansion apparatus. (1) and (2) Needle valve for CO_2 gas. (3) Brass expansion chamber tapped for the fittings. (4) Standard 3/8-inch brass pipe plug with cavity in face to seat embedded tissue. (5) Tissue in the clear resin with gelatin capsule removed.

alone for an equal period. To ensure removal of the alcohol they are then placed in two additional changes of monomer for at least one hour in each before embedding in the polymer.

Gelatin capsules serve as convenient embedding molds. The main body of the capsule is set upright in a wooden block or other base and filled with the monomer, to which has been added 1% (by weight) of a polymerization catalyst (2,4-dichloro-

benzoyl peroxide). After the tissue is placed in the mixture, the lid of the capsule is slipped on to retard evaporation. The assembled capsules are then suspended by strips of cellophane tape in an oven kept at a temperature of 45° to 50°C.

After 6 or 8 hours of heating, the monomer is polymerized into a solid matrix containing the tissue embedded at the bottom of the clear plastic. Several hours more at this temperature will ensure complete cure. The gelatin capsule may then be removed by soaking in water.

The embedded specimen is cemented into the mounting block with a mixture of pure gum rubber and paraffin. Then, with the thermal-expansion device clamped in the jaws of the microtome head, the entire assembly is cooled below room temperature (fig. 1 A). Upon the appearance of a thin layer of frost on the metal, the knife is adjusted so that the specimen just misses it on the cutting stroke. The specimen is then mechanically advanced at 2- or 3- μ increments until the first slice is made. At this point the mechanical advancing mechanism of the microtome is disengaged by setting it to zero and the gas flow reduced or stopped. The specimen then advances because of the thermal expansion of the metal holder. With a little experience the operator can soon judge the necessary time interval between cuts. Some control of the rate of specimen advance can be obtained by bleeding the carbon dioxide at various reduced rates into the expansion chamber.

Although polybutyl methacrylate has excellent cutting properties, the cut sections usually are found to be somewhat folded. They are lifted from the knife by a dry camel's hair brush, picked up with a dissecting needle, and placed on the surface of a 50% solution of dioxan in water, where they immediately flatten out. This flattening technique was first developed by Pease and Baker ('49). The sections are then floated onto water to remove the dioxan and those which are thin enough to show bright interference colors are then floated onto clean microscope slides and allowed to dry flat.

Sections prepared for phase-contrast microscopy are placed in acetone or toluene for about one-half hour to remove the

matrix and then mounted in Canada balsam. For ordinary light microscopy the matrix is dissolved and the section stained in the usual way.

In preparing material for the electron microscope, the sections are floated from the water onto clean glass slides and

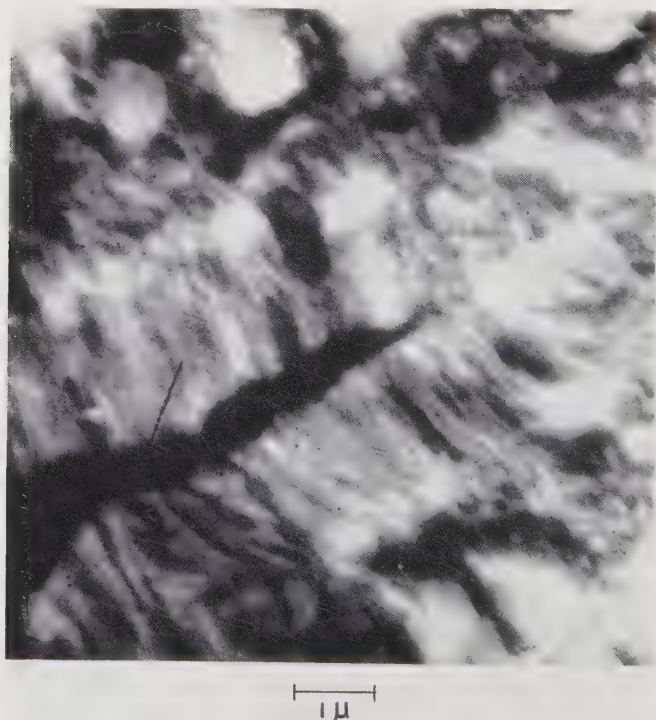


Fig. 2 Dog kidney, section through brush-border cilia of tubule lumen, fixed in Zenker-formol, chromium shadowed (5:1). Electron micrograph, total magnification $\times 10,400$ (el. $\times 5,200$, opt. $\times 2$).

dried flat. The matrix is then dissolved by placing the slide in acetone, toluene, or amyl acetate. A dilute solution of collodion in amyl acetate is allowed to flow over the slide bearing the tissue, which is then permitted to dry at room temperature. The collodion film is floated from the slide onto water, and electron-microscope specimen screens are placed

over the areas of the film containing the sections. The freely floating film, to which the specimen screens adhere, is removed from the water by flipping it over and out with a strip of short-fibered paper or a glass microscope slide.

The detection of structural details in some of the biological tissues sectioned at the Bureau was greatly improved by metallic shadow-casting in the manner of Williams and Wyckoff ('44). This process produces a three-dimensional as-

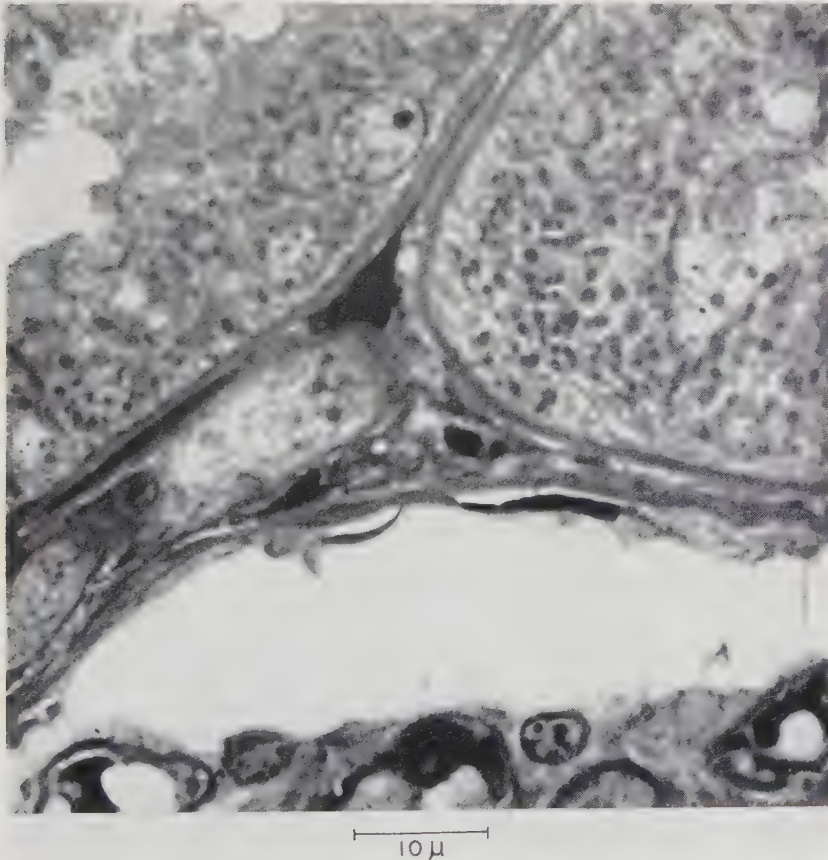


Fig. 3 Dog kidney, section showing tubule and part of glomerulus, fixed in Zenker-formol. Electron micrograph, total magnification $\times 2,600$ (el. $\times 750$, opt. $\times 3.5$).

pect as well as greater contrast in the structural details of some tissues (fig. 2). The sections are freed of the embedding media and stripped from the glass slide with a collodion film as described previously. They are then mounted, tissue side up, on the specimen screens and exposed to a metallic vapor under high vacuum. Material prepared for light microscopy may be stained and shadowed before mounting.

Although the new method for obtaining very thin sections has given excellent results, it possesses certain limitations. The greatest chance for failure appears to lie in the polymerization of the embedding mass. However, use of low-temperature catalysts and maintenance of a curing temperature at 45° to 50°C. will minimize difficulties at this stage. Occasionally tissues are injured during the polymerization reaction, but such tissues, which are easily detectable, can be promptly discarded. The tilt of the knife during sectioning is not particularly critical. Obviously the knife must be smooth, clean, and freshly stropped. Satisfactory edges are maintained by stropping on a flat strop which has been charged with diamond powder having a particle size of about 3 μ . On the whole, while fixation artifacts still remain problems for serious consideration, the new method makes possible the production of a high percentage of usable sections which have uniform thickness over a large area, and integrity of tissue structure (fig. 3).

ACKNOWLEDGMENT

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PLASMACYTES IN THE SPLENIC AND PORTAL VEINS OF HAMSTERS¹

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ONE FIGURE

The occurrence of many plasmacytes, or plasma cells, in the venous sinuses of the spleen, a site where lymphocytes and monocytes actively enter the blood (Maximow and Bloom, '48), suggested that the spleen might be the source of the plasmacytes that infiltrate the periportal connective tissue in the liver of sarcoma-bearing hamsters (Kelsall and Crabb, '47) and in pathological conditions, such as cirrhosis.

In the portal organs of the hamster, plasmacytes occur in the spleen, in the mesenteric and intestinal lymphoid tissue, and in the lamina propria of the stomach and intestines; however, the hamster resembles the rat and mouse in having more plasmacytes in the medullary cords of lymph nodes than are found in man (Maximow and Bloom, '48).

MATERIALS AND METHODS

The Syrian hamsters used in this work were from an inbred colony and comprised 7 controls, 16 pregnant, 6 lactating and 16 animals having transplants of a rapidly growing mixed-celled sarcoma that was produced in 1944 by injections, of 9,10-dimethyl-1,2-benzanthracene (Crabb, '46). The durations of pregnancy and postpartum periods were accurately determined from records of the exact time of mating because the gestation period of hamsters is one to two hours less than 16 days.

¹ This work was supported by the Beta Sigma Phi International Endowment Fund.

In order to determine the presence of plasmacytes within the terminal branches of the portal and splenic veins, sections of the spleen, pancreas and left anterior and cystic lobes of the liver were fixed in sublimate-alcohol and stained with toluidine blue, a combination of fixing and staining that has been found superior in demonstrating cytoplasmic basophilia. Additional

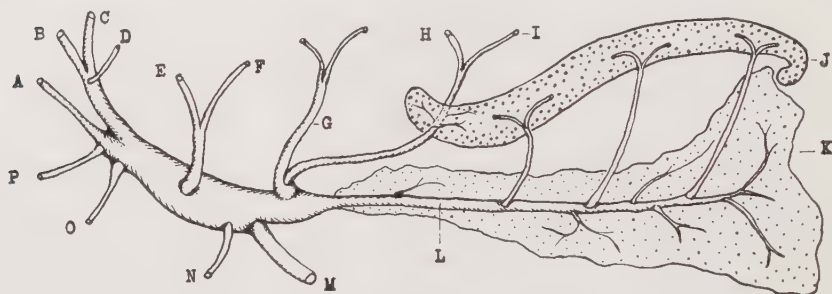


Fig. 1 Portal system of the hamster.

Schema of the portal system of the Syrian hamster viewed from the ventral side with the spleen everted craniad and both pancreas and spleen turned to the animal's left to expose the vascular relations of the two organs.

A, branch to right half of the cystic (right anterior) lobe of the liver; B, branch to the left half of the cystic lobe; C, branch to left anterior lobe; D, branch to caudate (Spigelian) lobe, which lies dorsal to the portal trunk; E, duodenal tributary; F, pyloric tributary; G, chief gastric tributary from both divisions of the stomach; H, gastric tributary from the right side of the fundus; I, gastric tributary from left side of the fundus (trunk vein does not perforate spleen in some individuals); J, tail, or ventromedial, end of the spleen (everted); K, ventromedial end of pancreas (everted; most of the diffuse part of the gland has been omitted); L, splenic vein; M, inferior mesenteric vein; N, tributary from the loop of the colon; O, branch to suprarenal (posterior caudal) lobe of the liver; P, branch to right anterior caudal lobe of the liver. (The right caudal lobe is divided into two distinct lobes and the left true caudal lobe is absent.)

staining methods included the Feulgen method for thymonucleic acid (Cowdry, '48), Pappenheim's methyl green-pyronin (McClung, '29), a test for ribonucleic acid (Stowell and Zorzi, '47), and Delafield's hematoxylin and eosin.

Leukocytes were counted in the venous sinuses in cross sections of the spleen, in the splenic vein as it passes between lobes of the pancreas (fig. 1, L), and in branches of the portal vein within the left anterior and cystic lobes of the liver.

The experimental animals were killed by cervical fracture and the erythrocyte and total white cell counts and dry smears were made immediately from flowing, jugular blood. In order to obtain sections of blood within the vessels, the postcava was ligated at the heart and at the common iliac branches, the abdominal viscera flooded *in situ* with a saturated solution of sublimate in 95% alcohol; then both the thoracic and abdominal viscera were removed and immersed in sublimate-alcohol (Cowdry, '48). The fixed blood vessels were dissected out, segments were embedded in rosin-celloidin-paraffin (Crabb, '49) and serially sectioned at 6 to 8 μ . By this method the blood was retained undisturbed in the lumen of the serially sectioned vessels so that differential counts in units of 100 were readily made from blood within several vessels of the same animal.

Plasmacytes of the hamster have the same structural and staining characteristics as those of man. In tabulations of sectioned vessels only cells having a considerable amount of basophilic cytoplasm were considered plasmacytes. Sections of plasmacytes passing through the nucleus and a small rim of cytoplasm could not be differentiated from lymphocytes and were, therefore, tabulated as lymphocytes.

To determine if the periportal infiltration observed in several pregnant hamsters which had one to three dead fetuses was due to absorption of necrotic substance, 1.5 cm³ of sterile emulsions of fetuses killed by asphyxia were injected subpannicularly into 8 adult male hamsters.

OBSERVATIONS

Plasmacytes were numerous in the blood sinuses of the spleen of hamsters in all experimental groups; however, the percentage of these cells progressively decreased as the blood flowed toward the liver and nearly reached zero in blood from the jugular vein. The highest mean percentage of plasmacytes, 24.8, occurred in the splenic sinuses of the 6 lactating hamsters (table 1). This percentage differs significantly from that of

TABLE 1
Percentage of plasmacytes in several veins and the differential leukocyte counts

GROUP AND NUMBER	PERCENTAGE OF PLASMACYTES			DIFFERENTIAL, JUGULAR			TOTAL WHITE	RBC
	Spleen	Splenic vein	P. V.	Jug.	Polym.	Lymph.	Mono.	
Control, 4								
Mean	13.0	2.3	3.0	0	20.5	74.3	5.3	
St. dev.	2.6	0.8	3.3		7.0	6.5	4.1	
Pregnant, 16								6 animals
Mean	14.8	9.2	4.6	0	31.9	60.7	7.4	5358
St. dev.	5.4	6.0	3.4		14.6	14.7	5.2	1254
Postpartum, 6								
Mean	24.8	9.0	3.8	0	32.3	61.2	6.5	
St. dev.	9.4	3.8	2.2		8.5	8.2	2.4	
Fetal inj., 8								6 animals
Mean	15.1	8.5	1.8	0	19.6	74.3	6.1	4874
St. dev.	10.0	3.7	2.0		3.5	5.1	2.3	1243
Sarcoma, 16								4 animals
Mean	16.1	9.8	4.4	0	67.8	24.7	7.5	9213
St. dev.	10.4	3.7	2.7		20.6	18.2	4.7	1965

P. V., portal vein; jug., jugular vein. The differential counts represent percentages; RBC are millions.

14.8 in the splenic sinuses of the 16 pregnant individuals; 13.0% in the control group; 16.1% in 16 hamsters having transplants of the sarcoma from 18 to 71 days; and 15.1% in the 8 males that received injections of triturated, dead fetal tissue and were killed three to 15 days later (table 1). Extramedullary erythropoiesis and myelopoiesis in the spleen of pregnant individuals did not alter the percentage of plasmacytes.

The percentage of plasmacytes in the portal vein was approximately the same in the tumor-bearing hamsters having extensive periportal infiltration of lymphocytes and plasmacytes as in the control and pregnant hamsters having no periportal infiltration. The average percentage of plasmacytes in the portal vein was 4.4 in the tumor-bearing, 3.8 in the lactating, 4.6 in the pregnant, 3.0 in the control and 1.8 in the fetal-tissue-injected group (table 1).

Blood from the jugular vein in each of the 5 above groups contained only an occasional plasmacyte, thus showing that none of the 4 factors considered was effective in altering the percentage of this cell in the peripheral blood. Elimination of the remaining plasmacytes that entered the splenic sinuses appears to be a function of the liver, for in the thoracic post-cava of a hamster having a transplant of the sarcoma for 41 days only 10 plasmacytes to 3,000 leukocytes were found. This is an incidence of 0.33%, while the average for the portal vein in the tumor-bearing animals was 4.4%.

The ratio of polymorphonuclear leukocytes to lymphocytes in the jugular blood of normal, pregnant and lactating hamsters was reversed in hamsters having the sarcoma. Blood from the jugular vein of 16 animals having a tumor duration of 18 to 71 days contained only 24.7% lymphocytes and 67.8% polymorphonuclears in contrast to 60.7% lymphocytes and 31.9% polymorphonuclears in the blood of the 16 pregnant animals; 61.2% lymphocytes and 32.3% polymorphonuclear leukocytes in the 6 postpartum hamsters; and 74.3% lymphocytes and 20.5% polymorphonuclears in the control hamsters. The neutrophilia in the tumor animals was coincident with leukocytosis (table 1).

Pregnancy and injection of fetal emulsions did not increase the number of plasmacytes entering the blood in the splenic sinuses or alter the total leukocyte count or the percentages of the groups of white blood cells which were considered (table 1).

Disintegration of plasmacytes. Several cytoplasmic and nuclear variations occurred in plasmacytes within the circulating blood, pycnosis, however, was the only stage in the disintegrative process that could be definitely established. The absence of cytoplasmic basophilia, like that which occurred when the thymonucleic acid increased during pycnosis, was due to a decrease in ribonucleic acid as indicated by the ribonuclease test (Stowell and Zorzoli, '47). Cytoplasmic vacuoles were more numerous in plasmacytes in the blood than in cells within lymphoid organs. A few plasmacytes in the blood had numerous small vacuoles similar to those described in Türk's irritation cells (Todd and Sanford, '27) and in "preplasmacytes" (Kracke, '41). Others contained one or several large cytoplasmic vacuoles resembling those in the description given by Böhm and von Davidoff ('01) of the first plasma cells observed by Waldeyer. Russell's bodies (Maximow, '28) have occasionally been observed in plasmacytes in lymph nodes and other organs but did not occur in the blood vessels in the spleen, portal vein or jugular veins in any of the groups considered.

The disintegration of plasmacytes in the blood appears to be a normal process since comparisons of control and tumor-bearing hamsters show no significant differences in the number entering the blood. The destruction of this cell in the hamster agrees with the statement of Maximow ('28) that "their ultimate fate seems always to be degeneration."

DISCUSSION

The occurrence of many plasmacytes in the venous sinuses of the spleen, the only lymphoid organ that is interposed in the blood stream (Maximow and Bloom, '48), may be significant in considering relations between plasmacytes and protein

metabolism. Cytoplasmic vacuoles and atypical vacuolization are considered to be evidence of protein secretion; but the question of whether all plasmacytes or only the medullary plasmacytes produce globulin has been posed (Leitner et al., '49). Bing ('40) expresses the idea that formation of globulin takes place in plasmacytes. Ehrlich et al. ('46), however, hold that plasma cells may produce beta globulin and related protein, but have not been found to produce gamma globulins.

A possible explanation for the presence of plasmacytes in periportal connective tissue of tumor-bearing hamsters is that these cells arise from lymphocytes in response to the presence of increased globulins in the blood. Janold and Wilter ('49) support this explanation by finding a correlation between an increase in plasmacytes and an increase in hyperglobinemia in the sternal bone marrow of patients with chronic hepatic insufficiency and conclude that plasmacytes may appear in response to increased globulin formation. A number of hematologists hold that plasmacytes arise from lymphocytes, but Parsons ('43) states that plasmacytes arise from fixed reticular cells in mice bearing methyleholanthrene and S37 transplanted sarcomas. Drinker and Yoffey ('37), however, state that "the one perfectly obvious fact is that the plasma cell arises from lymphocytes."

SUMMARY

1. Plasmacytes entered the circulating blood via the venous sinuses in the spleen of the Syrian hamster and represented 13.0% of the leukocytes in these sinuses in controls; 14.5% in 16 pregnant and 24.8% in 6 lactating hamsters; and 16.1% in 16 hamsters carrying sarcoma transplants for varying periods of time.

2. Plasmacytes disintegrated in the splenic and portal vein and were very rarely observed in blood from the jugular vein.

3. Infiltration by plasmacytes of the periportal connective tissue of tumor-bearing hamsters was independent of the number of plasmacytes entering the blood via the splenic

sinuses or contained in the circulating blood from the jugular vein.

4. Pregnancy and the injection of fetal tissue did not affect the number of plasmacytes or the ratio of leukocytes in the splenic sinuses, splenic vein and hepatic branches of the portal vein.

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THE EFFECTS OF ADVANCING AGE UPON THE HISTOLOGY OF THE OVARY, UTERUS AND VAGINA OF THE FEMALE GOLDEN HAMSTER (*CRICETUS AURATUS*)

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SIX FIGURES ¹

INTRODUCTION

There seems to have been little work done on the effect of age on the genital system of any of the laboratory forms prior to the beginning of the present decade. Even the work done in the past 10 years is limited.

In the human female (Hertig, '44) the first sign of aging in the ovary is an increased cortical gyration. Involution follows and there is a mild degree of stromal hyperplasia usually confined to the cortical region. As age advances germinal inclusion cysts form and increase in number, and some cortical granulomata may be present.

In the female rat (Wolfe, '43) aging results in a progressive decrease in the number of normal follicles, and an increase in the ratio of atretic follicles to normal ones. The ovaries of many old rats (Wolfe, Burack and Wright, '40) are characterized by "wheel cells" or "deficiency cells" similar to those found in the ovary of hypophysectomized rats. There is an increase in cystic follicles. The uterus and vagina have been reported to show an increase in collagenous connective tissue with age (Loeb et al., '38; Wolfe et al., '42).

¹ Acknowledgment and appreciation is hereby duly made to Mr. Albert H. Stenger who prepared the photomicrographs.

Up to the present there has been no work of this type done on the golden hamster. Thus it seems of some importance to consider this relationship to aging in this form. In view of the increasing popularity of the golden hamster (*Cricetus auratus*) as an experimental laboratory animal, it appeared to be of some importance to consider the relationship of aging in this form to any histological changes which may occur with time. This paper therefore concerns itself with the normal histology of the ovary, uterus and vagina of the golden hamster at various ages. The female hamster has been reported, by several workers, to attain an age of 24 months and to be sexually active from 2 to 11 months.

MATERIALS AND METHODS

The golden hamsters used in this study were part of a small colony maintained on a diet of mixed grain supplemented by dog chow pellets, cod liver oil and milk. Fresh greens were fed daily to provide the water necessary.

The ovaries, uteri and vaginae of 60 female hamsters killed at the ages of 1, 2, 3, 4, 6, 8, 9, 10, 12, 15, 18, 21 and 24 months were studied using various techniques. Animals which showed a reproductive cycle as determined by vaginal smearing were killed in the metestrous "A" phase of the cycle. The smear shown by older animals no longer running a cycle was similar in appearance to the metestrous "A" smear. Some of the animals had previously been mated and littered while others were virgins.

Some of the animals were first anaesthetized with Nembutal and then killed by cutting through the spinal cord at the base of the brain, others by carbon monoxide asphyxiation.

The tissues were fixed in either Bouin's or Zenker's fluid. The ovaries were cut serially at 6 μ . Representative sections of the uteri and vaginae were also cut at 6 μ and stained in the same manner as the ovaries.

Ovaries, uteri and vaginae were subjected to a complete histological examination. They were stained with Galigher's ('34) modification of Harris' haematoxylin and eosin; Mas-

son's trichrome technique for connective tissue; or Kühne's technique for hyaline degeneration (Carleton and Leach, '38).

In connection with the study of the ovaries, quantitative methods were used as much as possible. Both normal and atretic follicles, and corpora lutea were counted in one ovary of each hamster. All sections were examined and the follicles counted at the level at which the ovum was observed. Determinations of follicles' size were also made. In cases where the follicle was not spherical, the diameters of the length and the width were measured and the average used for comparison with other follicles. Follicles, both normal and atretic, whose average diameter was less than 0.2 mm, and corpora lutea of an average diameter less than 0.3 mm were not recorded.

The average diameter of the uteri at various ages were measured. The measurements were made at approximately the midpoint of the length of the uterus.

OBSERVATIONS

The following description of the three-month-old hamster ovary may be considered as a normal picture. All animals between three and 9 to 10 months showed a similar structure.

A. Ovary

The cortex and medulla of the golden hamster ovary blend into each other with no distinct line of demarcation. The medulla is composed of a stroma of loose connective tissue containing many blood vessels and blood sinuses, nerves and lymph vessels. A few smooth muscle fibers are seen distributed throughout.

The cortex is more compact. It is covered by a thin outer layer of mesothelium closely applied to the germinal epithelium. The cells of the germinal epithelium are cuboidal with large irregularly oval vesicular nuclei. The tissue between the follicles is richly cellular and blood vessels are present but no large blood sinuses are seen. The interstitial tissue cells are elongated or oval with vesicular oval nuclei. The stroma im-

mediately below the epithelium is more densely fibrous with fewer cells and more fibers.

The primary follicles range in size from about 0.035 to 0.067 mm in diameter. For the most part they are located at the periphery of the gland immediately beneath the germinal epithelium. In a few cases they are found slightly deeper in the cortex. They are not regularly arranged along the edge of the ovary but rather in groups or clusters at various levels. The follicles lie directly in the stroma with no connective tissue capsule. The follicle cells are flattened or cuboidal with oval vesicular nuclei.

The growing follicles lie deeper within the cortex and their diameter varies from 0.07 to 0.36 mm. The ovum contains a large vesicular nucleus with a deep staining chromatin reticulum and a granular cytoplasm. It is surrounded by several layers of cuboidal follicle cells. In several cases atretic follicles were seen containing ova which showed two nuclei with no division of the cell body.

The Graafian follicles, with an average diameter of 0.4 mm, are located at or near the periphery of the gland. The cytological structure resembles that of most other mammalian forms.

The corpora lutea range from about 0.3 to 0.6 mm in diameter and are formed by cords of cells extending radially from the center to the periphery. Groups of blood cells are seen between the cords of cells. In some cases the boundaries of the corpora lutea are not distinct and they may be confused with atretic follicles.

The size of the atretic follicles varies similarly to the normal ones. The zona pellucida appears folded or wrinkled. The follicle cells form cords and gave an appearance comparable to that of the corpus luteum.

Table 1 and figure 1 give the number of normal growing and atretic follicles, and corpora lutea found per ovary during various portions of the life span. The numbers of normal and atretic follicles were also combined to give the total number of

follicles per ovary. Table 1 and figure 1 show the ratio of atretic to normal follicles.

Normal follicles are most abundant in the 6-month-old hamster. However, the level is about the same from two to 9 months. There is a slight decrease in the one month animals. In succeeding groups there is a progressive decrease in the average number of normal follicles per ovary. This loss becomes pronounced at 10 months and drops gradually up to 15 months of age. Thereafter the drop is rapid up to 24 months.

TABLE 1

MOS. OF AGE	NO. ANIMALS COUNTED	FOLLICLES (AV. NO./OVARY)			CORP. LUT.	RATIO ATRETIC TO NORMAL
		normal	atretic	total		
1	5	38.00	17.40	57.40	2.4	0.46
2	5	42.60	17.20	59.80	3.0	0.40
3	5	42.80	11.60	54.40	4.8	0.27
4	5	43.20	16.40	59.60	8.8	0.38
6	5	45.60	21.40	67.00	6.6	0.47
8	6	44.50	22.00	66.50	5.8	0.49
9	5	41.60	25.20	66.80	5.2	0.61
10	5	36.40	19.20	55.60	8.0	0.53
12	6	33.34	26.67	60.01	3.2	0.80
15	5	32.60	23.60	56.20	4.2	0.72
18	5	19.00	16.80	35.80	4.8	0.88
21 ¹	5	12.40	15.40	27.80	3.0	1.24
24 ²	2	10.00	20.00	30.00	5.0	1.50

¹ One ovary cystic in this group.

² Of the 10 ovaries in this group 6 were cystic and one both tumorous and cystic. Therefore only two were counted.

The average number of total follicles is greatest between 6 and 9 months of age. Between one and 4 months the level is lower but consistent. There is a rapid rise up to 6 months. There is a sharp drop between 9 and 10 months; a rise again at 12 months and then a progressive decline in older groups.

The atretic follicles show a high value at one and two months; a drop at three months, followed by a rise up to 9 months, then a drop between 9 and 12 months. At 12 months the average number is high again and then there follows a gradual decline.

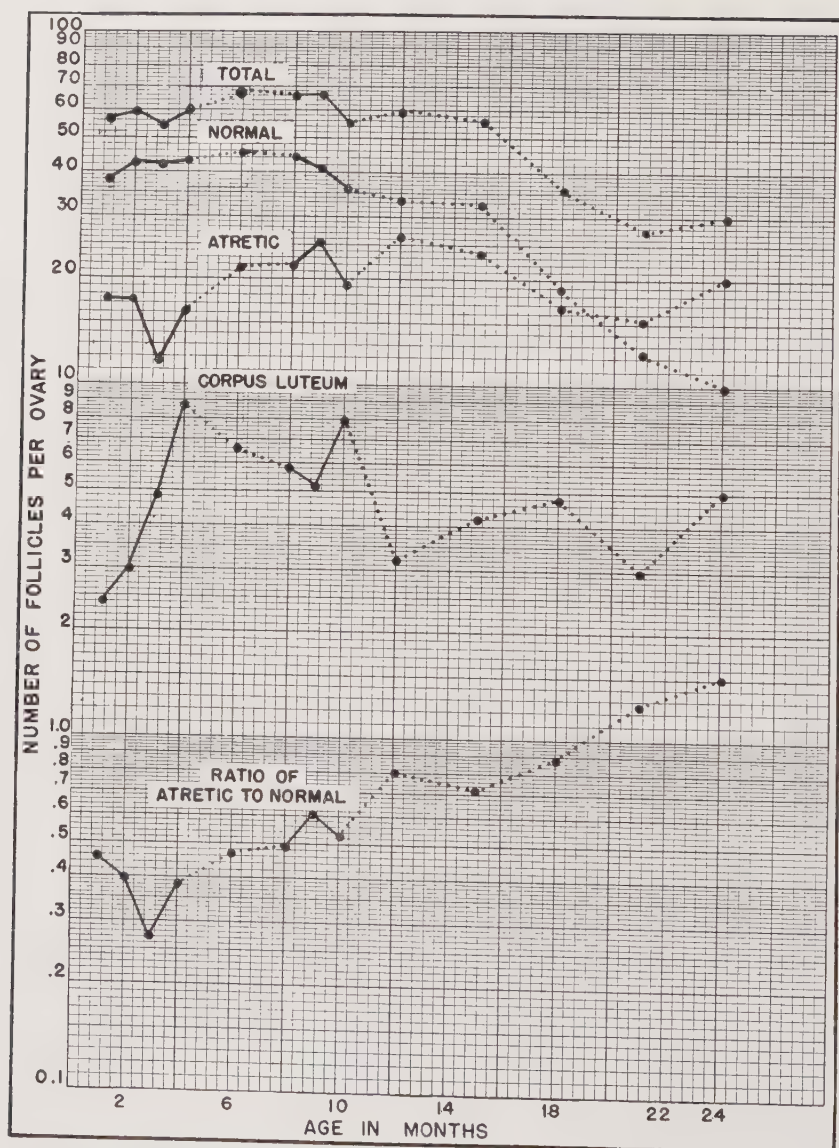


Fig. 1 Semi-logarithmic graph showing effect of advancing age on the average number of total follicles, normal follicles, atretic follicles, corpora lutea, and the ratio of atretic to normal follicles in the ovary of the hamster; age in months on the abscissa and number of follicles per ovary on the ordinate.

The average number of corpora lutea increase rapidly from one to 4 months. The curve (fig. 1) then drops up to 9 months of age. The average number increase again at 10 months and show another reduction at 12 months. Up to 18 months of age there is a slight rise and finally a progressive decline to 21 months at which time there is another increase.

Those sections stained with Kühne's technique for hyaline degeneration show crystal violet staining in some of the blood cells and the chromatin of a few nuclei of the interstitial cells scattered throughout the gland. There seems to be no particular change with advanced age and no hyaline degeneration was noted.

From 9 to 10 months of age on there is a marked connective tissue invasion of the organ. Connective tissue fibers are found in the medulla, the outer part of the cortex, and surrounding some of the follicles.

By 15 months the stroma of the medulla becomes looser. cortical stroma is also less dense by 18 months.

At 21 to 24 months of age the amount of fibrous connective tissue present has greatly increased. With the Masson technique it is seen that the fibers of the cortex are quite fine. Most of them are located in the outer cortex and around the follicles while there are relatively few in the inner cortex. The fibers of the medulla are coarser and more numerous. By 24 months of age very few follicles, either normal or atretic, are present (fig. 4). There also seems to be a greater increase in medullary than in cortical tissue.

Very old hamsters, 21 to 24 months of age, also show a tendency to develop cystic ovaries or, in one case, a tumor of the ovary plus a cystic condition of the same gland. In two cases only one ovarian cyst was found; the other ovary appearing normal. In three cases the cysts were bilateral.

B. Uterus

The uterus of the hamster is composed of the usual two parts, endometrium and myometrium. The surface epithelium is simple or pseudostratified. In a few cases some mucous and

slough-off cells are seen in the elongated lumen. Just below the epithelium (lining) there is a fine layer of connective tissue fibers which run parallel to the surface. The tissue beneath the epithelium contains a few simple tubular glands, serous in nature. The stroma is made up of thin connective tissue fibers and cells. The inner portion is highly cellular with connective tissue fibers between the cells. The outer part is less cellular and contains more fibers.

The myometrium contains two layers of smooth muscle, an outer longitudinal band and an inner circular one. Between the two bands there is a connective tissue stroma containing many blood vessels and sinuses. The connective tissue fibers between the muscle bands are thicker than those of the endometrium. The outer longitudinal muscle layer extends into the mesometrium and is there replaced by loose connective tissue. The circular muscle band is thinner in the distal and proximal ends of the uterus than in the central portion. A thin layer of adventitial cells is present on the outside.

The histological structure just described is typical for the one- to 4-month-old animals (fig. 5). Between 6 and 15 months there is a progressive increase in connective tissue between the fibers of the circular muscle layer. The fibers of the stroma increase in abundance, forming a network with the cells in the interstices. However, the subepithelial stroma is still more cellular than the outer portion.

In the 18 and 21 month groups the outer stroma fibers appear thicker and more dense. There seems to be a decrease in the amount of circular smooth muscle while the longitudinal muscle is about the same.

By 24 months there is a more definite reduction in the circular muscle. The longitudinal muscle is still well preserved. The fibers of the outer stroma are very coarse. The inner stroma also has more connective tissue, but here the fibers are finer (fig. 6).

The diameter of the uterus (table 2, fig. 2) shows a rapid increase up to three months; then a plateau to 6 months and

TABLE 2

MOS. OF AGE	NO. OF ANIMALS COUNTED	DIAMETER OF UTERUS <i>mm</i>
1	5	1.54
2	5	1.72
3	5	2.38
4	5	2.38
6	5	2.36
8	6	2.66
9	5	2.98
10	5	2.66
12	6	2.94
15	5	2.97
18	5	3.05
21	5	2.98
24	5	3.15

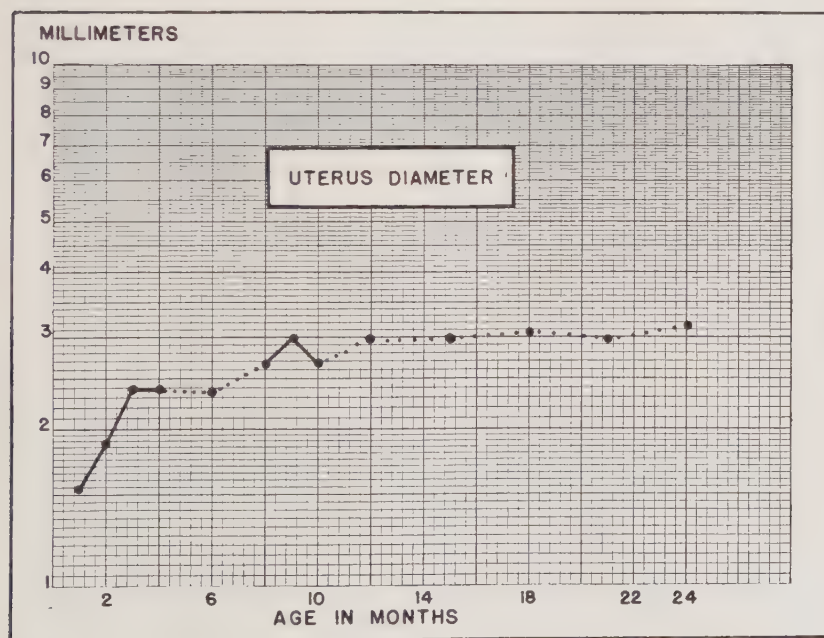


Fig. 2 Semi-logarithmic graph showing effect of advancing age on the average diameter of the uterus of the hamster; age in months on the abscissa and width in millimeters on the ordinate.

finally a gradual rise up to 24 months with the exception of a drop at 10 months.

C. Vagina

The epithelium of the vagina is stratified squamous. The lumen sometimes contains mucous lining the epithelium but this condition seems to have no relation to age and is probably connected with the reproductive cycle. The stroma is made up of loose connective tissue containing blood vessels, a few leucocytes and an occasional isolated lymph node. It is highly cellular. No glands are present. The smooth muscle layer is thin and composed chiefly of longitudinal fibers with a few circular fibers mixed with them. The muscle bands are not continuous but interrupted by connective tissue. The fibrosa is composed of dense connective tissue.

The previous description is typical of the one- to 4-month-old hamsters. With advancing age the cellular content of the stroma becomes gradually reduced. From 6 months on there is a progressive increase in the thickness and density of the connective tissue fibers. Most of the fibers are circular in their distribution, however, in a few cases, some seemed to be radial or oblique in their course.

No mucification or hylainization were noted.

DISCUSSION

A. Ovary

The ovary of the golden hamster resembles, for the most part, that of other forms. The ova containing two nuclei without a cytoplasmic division have also been reported in this form by Ward ('46) who claims that such divisions may represent early degeneration of the follicle. Harman and Kirgis ('38) report ovum degeneration, in the guinea pig, as beginning with several nuclear and cytoplasmic divisions, and therefore this condition may merely represent the beginning of atresia as no case of a two cell stage in the unfertilized ovum has been published.

The corpora lutea present at the metestrous "A" stage of the cycle are those formed during preceding cycles and therefore are becoming reduced in size and resorbed thus accounting for the indistinct boundaries in some cases.

The relative increase in the medulla over the cortex with advancing age was also described for the human ovary (Hertig, '44).

No difference in counts was noted between those animals which had previously littered and those which were virgins. The animals were killed throughout the year, thus it is possible that some of the variations are due to seasonal, temperature or humidity differences. A progressive decrease in the average number of normal follicles and also total follicles is comparable with Wolfe's ('43) findings for the rat, and with Loeb's ('41) for the mouse. Hertig ('44) reported a similar gradual loss of ova in the aging human ovary. A statistical analysis of our figures indicates a significant difference in normal follicles between one and two months; 15 and 18 months; and 18 and 21 months. The atretic follicle counts are statistically significant between two and three months; three and 4 months; 9 and 10 months; 10 and 12 months; and 15 and 18 months. Thus the variation in the average number of atretic follicles was more irregular and as far as absolute number is concerned, an increase associated with advanced age cannot be clearly established. Thus the ratio of atretic to normal follicles was calculated (fig. 1) and from this can be seen a definite and rapid increase in old hamsters. The ratio in one-month-old animals is high and then decreases up to three months. This may be due to the fact that in these young animals many of the normal follicles are below the size range counted, i.e., average diameter 0.2 mm.

The curve showing the variation of the corpora lutea is highly irregular. The first peak at 4 months is probably the result of the recent attainment of sexual maturity. The subsequent peaks and troughs cannot be explained on the basis of this work. It should be noted however, that the peaks, and likewise the troughs, become progressively lower with advanc-

ing age. The only differences which are statistically significant are those between the two and three month; three and 4 month; and 10 and 12 month groups.

It will be noted that the curves for total and atretic follicles show a marked drop at 10 months followed by a rise (fig. 1). A similar situation is also found in the curve showing the average diameter of the uterus (fig. 2). It is possible that this may be explained on the basis of some seasonal periodicity. All the animals in this age group were killed during March and April, and while no particular breeding season was noted in this colony, the litters from February to March or April were sometimes smaller. Other workers have reported similar variation in litter size. However, of all the changes noted only the change in atretic follicle numbers has been found to be significant statistically for the number of animals used in the current observations.

No hyalinization of blood vessels such as reported by Loeb ('41) for mice was seen. This seems to indicate that the hamster is similar to the rat in this respect (Wolfe et al., '42).

Recent work has been reported (Kaunitz and Slanetz, '49; P'an et al., '49) in which it was found that a diet containing synthetic dl alpha tocopherol increased the percentage of pregnancies in old female rats which had become sterile, and caused a restoration and shortening of the estrous cycle. More than 50% of the animals maintained on this diet gradually developed a menopause condition associated with irregularity and finally absence of a cycle, resulting in sterility (Kaunitz and Slanetz, '49). These authors claim that this may not necessarily be a consequence of natural aging because tocopherol can restore the cycle and fertility, but rather that the minimum requirement of tocopherol is increased steeply with age. There is a need for more work along these lines particularly in relating vitamin E to hormone production and activity.

B. Uterus and vagina

Loeb et al. ('38, '39) and Loeb ('41) reported an increase in fibrillar connective tissue and hyaline deposition in the stroma

of the mouse uterus and vagina. It was found by Suntzeff and his associates ('40) that estrogen hastened and intensified these results and that they were reversible when estrogen administration was stopped. Similar results were found in studies on the rat and it was also found that they were more marked in virgins (Burack et al., '40).

Loeb et al. ('38), studying the mouse, and Wolfe et al., ('42), in the rat, found that the transformation of connective tissue from a reticular to a collagenous type took place in the first few weeks of life and progressed more definitely in older animals especially after the sexually active period.

The results of this study show a similar chain of events occurring in the hamster. Since reticulum stains were not done it is impossible to say whether the connective tissue described is of the reticular or collagenous type.

The changes in the diameter of the uterus are similar to those described by Wolfe et al. ('42) for the rat.

The increased hyalinization noted by Loeb ('41) in the mouse was not found to be true for the hamster. Wolfe et al. ('42) also failed to find such deposits in the rat.

No mucification of the vaginal epithelium was found in these animals such as is described by Meyer and Allen ('32) for the guinea pig, mouse and rat and also by Wolfe et al. ('42) for the rat.

CONCLUSIONS

1. The general histology of the ovary, uterus, and vagina of the golden hamster resembles that described for other forms.

2. In the ovary there is a progressive decrease in the average number of normal and total follicles and corpora lutea, and a definite increase in the ratio of atretic to normal follicles associated with advancing age.

3. A relative increase in the medulla over the cortex was found with old animals.

4. No hyalinization was noted in the aging ovary, uterus or vagina.

5. The changes with age in the uterus include a gradual and progressive increase in abundance, thickness and density of connective tissue fibers.

6. The circular smooth muscle of the uterus seems to atrophy with advanced age. The longitudinal smooth muscle maintains a state of good preservation.

7. The uterus increases in diameter with advancing age.

8. The changes with age in the vagina are similar to those in the uterus. There has been found a gradual reduction of the cellular content of the stroma, and a progressive increase in the thickness and density of the connective tissue fibers.

9. The changes associated with advancing age in the ovary, uterus and vagina of the golden hamster are similar, though not identical, to those found in the mouse and rat.

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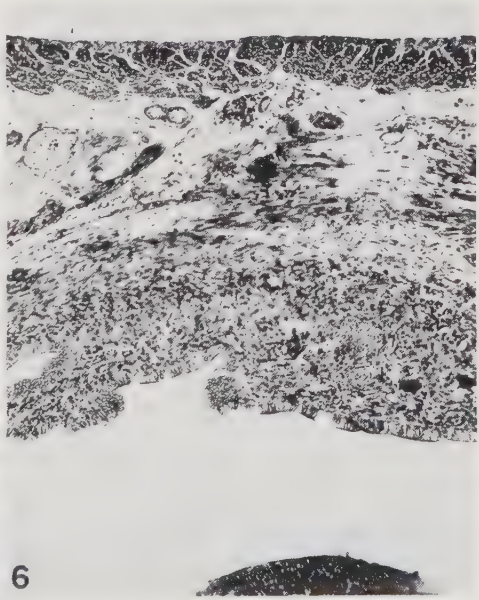
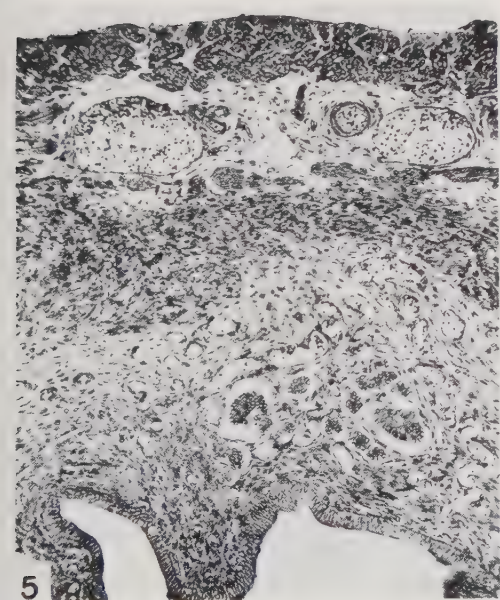
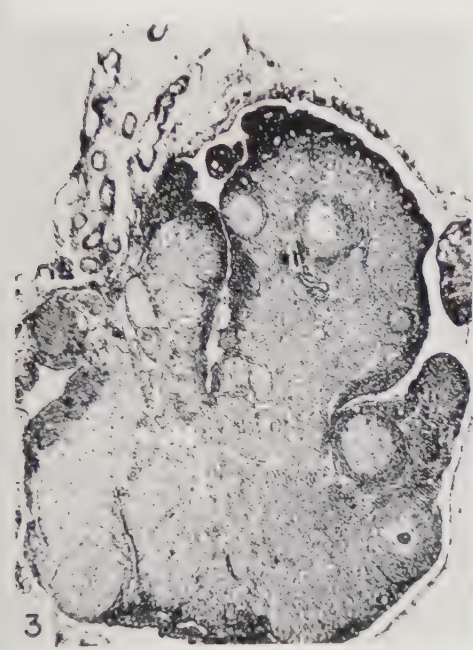
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PLATE 1

EXPLANATION OF FIGURES

All figures are photomicrographs. Sections are 6μ thick.

- 3 Ovary of a three-month-old hamster. Zenker, hematoxylin and eosin. $\times 36$.
- 4 Ovary of a 24-month-old hamster, showing one follicle and extreme connective tissue infiltration. Zenker, hematoxylin and eosin. $\times 31$.
- 5 Cross section, uterus of a 3-month-old hamster. Zenker, Masson. $\times 96$.
- 6 Cross section, uterus of a 24-month-old hamster, showing a decrease in the width of the circular muscle band and some increase in the connective tissue of the stroma. Compare with figure 5 and note the over-all decrease in uterine wall effecting both the endometrium and myometrium. Zenker, Masson. $\times 96$.



THE LINING OF THE ALVEOLI IN MICE, RATS,
DOGS, AND FROGS FOLLOWING ACUTE
PULMONARY EDEMA PRODUCED
BY ANTU POISONING¹

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FOURTEEN FIGURES

INTRODUCTION

The late W. S. Miller ('47) stoutly maintained during many years' study that the pulmonary alveolus in mammals is lined by a continuous membrane of flattened cells of endothelial origin. However, he was unable to demonstrate such a membrane lining the alveoli in normal lungs of animals and man. Employing pathological material from the lungs of individuals dying of lobar or interstitial pneumonia or of mitral heart disease, Miller ('32, '47) observed certain membranes which he considered to be pulmonary alveolar epithelium and accepted these findings as evidence for a continuous epithelium in the normal lung. In his studies, Miller ('23, '25, '32, '47) emphasized the part played by the serous exudate, which was considered to loosen and separate the so-called epithelial membrane from the alveolar walls, thereby making it more readily demonstrable. The production of acute pulmonary edema by alpha-naphthyl thiourea provides a new method for the experimental study of the nature of the lining of the pulmonary alveolus, and offers a particularly

¹ Aided in part by the Douglas Smith Foundation for Medical Research, the University of Chicago. Presented in part at the 61st Annual Session of the American Association of Anatomists, Madison, Wisconsin, April 22, 1948.

good opportunity to evaluate the views of Miller on this subject.

The edema-producing property of thiourea has been known for a long time (Binet, 1893). More recently, Richter and associates ('41, '42, '45, '46) have studied the toxic effect of a variety of thiourea compounds. Alpha-naphthyl thiourea (hereafter referred to as ANTU) was found to be especially toxic for a variety of laboratory animals (Richter, '45; Dieke and Richter, '46; Anderson and Richter, '46; and McClosky and Smith, '45). The characteristic finding in animals dying of acute poisoning was marked pulmonary edema and pleural effusion. Drinker ('45) and Latta ('47), studying the mechanism of action of ANTU in dogs and rats, concluded that it acted specifically on the pulmonary capillary endothelial cells to increase their permeability, which resulted in filling the air spaces with edema fluid. Drinker and Warren ('43) discuss the genesis and resolution of pulmonary transudates and exudates from other causes.

The gross and microscopic changes occurring in the lungs of animals (rats and dogs) dying of acute ANTU poisoning have been described by Drinker ('45) and Latta ('47). They refer to no specific histological changes which occur in the alveolar walls of the edematous lungs. In our study, special attention has been given to the histological changes in the lungs following acute ANTU poisoning. As the air spaces of the frog (*Rana pipiens*) are lined by a flattened, histologically-demonstrable epithelial membrane (Loosli, '38), this animal was employed as a control of the method and as a basis for interpreting the findings in the mammalian lungs.

MATERIALS AND METHODS

The animals employed in this study were apparently healthy adults. The number of frogs, mice, rats, and dogs employed as test and control animals, the dosage of ANTU, and the approximate survival time are given in table 1.

Following the intraperitoneal injection of ANTU, the animals were given food and water and observed at frequent

intervals. When moribund, they were sacrificed by the intraperitoneal (frogs, mice, and rats) or intravenous (dogs) injection of pentobarbital sodium. The chest cavity of an animal so sacrificed was immediately opened, and a ligature placed about the base of the heart to keep the blood in the pulmonary vascular system. The lungs were then expanded slowly by the intratracheal injection of Zenker-formol fixing solution, removed in toto and immersed in the fixative for 8 hours (Loosli, '35, '37, '38). The lungs of two animals of each of the test and control groups were perfused with 10% formalin, followed by the intratracheal fixation with the same solution. After fixation, specimens of the lungs were dehydrated, embedded in paraffin, sectioned and stained with Maximow's hematoxylin-eosin-azure II and Mallory's azan techniques (Buchsbaum and Loosli, '36).

TABLE 1

SPECIES	TEST	CONTROL	MG ANTU/KG BODY WT.	SURVIVAL TIME
				<i>hrs.</i>
Frogs	19	4	8000-9000	2-30
Mice	36	3	100-200	2-12
Rats	14	4	10-50	4-8
Dogs	5	2	10-40	8-26

OBSERVATIONS

Although the animals were all sacrificed in the moribund state, it was apparent that the survival time decreased with the administration of the larger doses of ANTU. The frogs became unresponsive and lethargic and some of those given large doses of ANTU died as early as two hours after injection. Mice and rats gradually became inactive. Respiratory movements became rapid and labored and, when the animal was moribund, edema fluid exuded from the nares. In some rats, rhonchal breathing could be heard. The dogs showed no gross evidence of ANTU toxicity until two to three hours before death. The first sign of injury was an increase in respiratory activity and coughing. Dyspnea gradually became

more marked, even at rest. Terminally, a rhonchal breathing could be heard without the aid of a stethoscope, and white frothy fluid dripped from the nose.

Frog lungs from normal animals. The frog lung is a sac containing an axial airway into which numerous large, wide-mouthed alveoli open (fig. 1). About the mouths of alveoli, the airway is lined by a pseudostratified columnar ciliated epithelial membrane. The transition from cuboidal to squamous epithelial cells as the membrane extends over the capillary surfaces is abrupt (fig. 2). The alveolar wall is composed of a central septum of connective tissue containing elastic and collagenous fibers and smooth muscle cells. On each side of the central septum is a network of capillaries (fig. 1). Lining the alveolus is a flattened epithelial membrane (figs. 2 and 3). The epithelial cells are large and their nuclei, in groups of two to 4, lie in the intercapillary spaces. The cytoplasm of the cells extends as a thin membrane over the surfaces of the capillaries. That portion of the cytoplasm immediately about the nucleus may be vacuolated. The blending of this portion of the cytoplasm with that which covers the capillaries can readily be observed in thin sections (figs. 2 and 3). The outlines of the large flattened cells can be demonstrated clearly in thick sections with the silver nitrate technique (Loosli, '38).

Frog lungs from ANTU treated animals. Grossly, the lungs are wet and edematous. Microscopically, the lymph spaces and blood vessels of the septa are dilated. There is also interstitial edema of the septa. The capillaries and blood vessels are congested and there is serous precipitate in the air spaces (fig. 4). The epithelial membrane is separated as vesicles (fig. 4) or sheets (figs. 5 and 6) from the alveolar surfaces. Where the displacement of the cells is slight, the nuclei, which formerly lay between loops of capillaries, hang by a pedicle from the squamous portion of the cytoplasm. In such areas, the detached epithelial membrane preserves the contour of the capillaries (fig. 6). In spite of the separation of the overlying epithelial membrane by the edema fluid,

the blood capillaries remain intact (figs. 5 and 6). The denuded endothelial cell surface appears smooth and thin but not thinner than the membrane separating the capillaries from the air spaces in the lungs of normal mice, rats, and dogs seen in ordinary thin sections (compare figs. 5, 6 and 8).

Rat lung from normal animals. Because of the similarity in microscopic structure of the normal lungs of the rat, mouse, and dog, only a description of the respiratory portion of the rat lung will be given. The cuboidal epithelial membrane ends abruptly at the junction of the terminal respiratory bronchioles with the alveolar ducts (fig. 8). The walls of the alveolar ducts and alveoli are highly vascular (figs. 7 and 8). An alveolar septum consists of a mesh of collagenous, reticular and elastic fibers. The capillaries form a single network and are supported by this mesh so that they are exposed on both sides of the septum. The exposed capillaries appear to be covered only by the ground substance, which encloses also the elastic and reticular fibers. In some of the intercapillary spaces are septal cells (fig. 8). These cells may occupy various positions in and on the alveolar walls. The most characteristic position is at the interstices of the septa. The cytoplasm is slightly basophilic and abundant and may extend across a septum and protrude into the adjacent alveolar spaces. The cytoplasm of the septal cell never is seen to extend as a thin membrane over the capillary surfaces. A detailed description of the septal cell in normal mammalian lungs has previously been made by one of us (Loosli, '38).

Rat lungs from ANTU treated animals. Grossly, the lungs are wet and boggy. The edematous areas appear purplish-red in color and often involve the greater portion of the lungs. A thin white frothy fluid fills the trachea, and fluid of a similar character, though not frothy, is present in the pleural cavities. In sections, the edematous exudate appears as a serous or protein precipitate which is present in alveoli, alveolar ducts, and smaller bronchioles (fig. 9). The amount of serous precipitate varies in different parts of the lung. In some areas in the exudate, a fine meshwork of fibrin is

present which extends from one alveolus to another through the pores of Kohn (fig. 10). Here and there a few cells are also present in the exudate. These are free pigment-filled macrophages, polymorphonuclear leucocytes, lymphocytes, and monocytes. (fig. 10).

In the edema-filled lungs, the alveolar septa do not appear to be altered. The capillaries are congested with red blood cells and leucocytes, but there is no hemorrhage or diapedesis of red blood cells into the air spaces. The septal cells are present in their characteristic positions on the alveolar walls and are not detached by the exudation of serous fluid into the alveoli (fig. 10). Filaments of cytoplasm of these cells are not seen to be lifted from the surfaces of the capillaries, as occurred in the lungs of the ANTU treated frogs. Likewise, non-nucleated plaques are not demonstrated in the lungs of the ANTU treated animals (fig. 10). The endothelial membrane of the capillaries does not differ perceptibly from that in the lungs of normal rats (compare figs. 8 and 10).

Mouse lung from ANTU treated animals. Grossly, the lungs of mice treated with ANTU show extensive areas of edema which appear similar in color to that seen in the lung of the rat. Microscopically, the findings are also similar (figs. 11 and 12). Fibrinous precipitate, however, appears to be less pronounced in the exudate of the lung of the mouse. Where it is present, strands of fibrin can be seen passing from one alveolus to another through the pores of Kohn (fig. 12). As in the rat lungs, the septal cells are neither altered in appearance nor detached from the alveolar walls (fig. 12), nor do they show processes lifted by the edema fluid from adjacent capillary surfaces as was noted in the lungs of ANTU treated frogs. Non-nucleated plaques are not demonstrated in the exudate or on the alveolar surfaces (fig. 12). There is no demonstrable change of the capillary endothelium or of the alveolar septum.

Dog lungs from ANTU treated animals. Marked edema is present in the lungs of all the animals. The edematous areas are purplish-red in color and diffusely distributed

throughout the lungs. Thin, watery, serous fluid exudes from the nares of the dogs when they are placed on the autopsy table. The pleural and pericardial cavities contain large amounts of serous fluid. Frothy serous fluid fills the trachea. In one animal, there is also extensive edema of the mediastinum and pleura. Microscopically, the air spaces of the lungs are seen to be filled with edematous precipitate (fig. 13). As in the case of mice and rats, there is marked edema of the vessel walls and congestion of the lymphatics (fig. 13). The bronchial surfaces are also covered by serous precipitate. The apparent lack of edema fluid in the larger bronchial passages may be explained on the basis of the intratracheal and intrabronchial method of fixing the lungs.

As was also seen in the mouse and rat lung of ANTU treated animals, the alveolar exudate in the dog lungs contains fibrin, strands of which pass through the pores of Kohn from one alveolus to another. There are a few cells in the exudate. These are polymorphonuclear leucocytes, lymphocytes, and monocytes from the blood (fig. 14), and an occasional free pigment-filled macrophage. The septal cells are not detached by the exudation of edema fluid (fig. 14). As in the lungs of normal dogs, they can be seen in various positions in and on the alveolar walls (fig. 14). Cytoplasmic extensions of these cells are not observed to be separated from the capillary surfaces. Non-nucleated plaques likewise are not observed in the lungs of ANTU treated dogs. The membrane separating the capillary lumina from the air spaces appears not discernibly different from that in the lungs of normal animals. There is no perceptible thickening or edema of the connective tissue of the alveolar septa.

DISCUSSION

The use of ANTU provides an excellent experimental tool for producing acute pulmonary edema in experimental animals (Richter, '42; Drinker, '45; and Latta, '47). The lymph flow, the composition of edema fluid, the hemodynamics, and the probable mechanism of action following ANTU poison-

ing in the dog are described by Drinker ('45) and Latta ('47). The observations in this study on the gross and microscopic appearance of the edematous lungs of treated dogs, rats, and mice are similar to those recorded by these authors. The characteristic findings were marked edema of the walls of the arteries and veins, especially at the hilum, congestion of the pulmonary lymphatics, and a relatively acellular edematous exudate in the pulmonary air spaces. It seemed of interest to us, as Latta ('47) has shown, that in spite of the marked amount of edema fluid in the air spaces, the bronchial walls showed essentially no reaction, except at the hilum. This is apparently due to congestion of lymphatics that lie in the adventitia and follow the course of the arteries and veins to the hilum.

As was noted by Latta ('47), no thrombi in the pulmonary arteries, veins, or capillaries were observed; nor was there discernible destructive change in the walls of the vessels and capillaries of the ANTU treated animals.

The cells in the alveolar edema fluid of mice, rats, and dogs were similar to those seen by Drinker ('45) and Latta ('47). There were in varying numbers, free pigment-filled macrophages (normally present in the air spaces), lymphocytes, monocytes, and polymorphonuclear leucocytes. In no animal, regardless of the length of survival following ANTU administration, were the cells in the exudate conspicuous. These observations are also similar to those of others (Farber, '37; Henneman, '46) who have studied acute pulmonary edema produced experimentally by different methods.

Drinker ('45) and Latta ('47) make no reference to possible cellular changes that may have occurred in the alveolar walls. Drinker holds the view of Miller that the alveoli are lined by a continuous thin, plate-like epithelial membrane. As has previously been stated, Miller ('47) was unable to demonstrate such a membrane in normal lungs of animals and men. His concept of the structure of the alveolar wall in mammalian lungs has been determined principally from the examination of pathological material from the lungs of men. Although

illustrations of Miller ('32) do show a detached cellular membrane, his conclusions concerning the lining membrane in normal lungs are open to question, because he employed pathological material.

Parker and Weiss ('36) have shown that chronic congestion of the pulmonary vascular bed in cases of mitral heart disease results in marked histological changes in the alveolar walls. There is extreme thickening of all the elements, particularly of the central stroma of the alveolar walls. There is an increase in the amount of collagenous, reticular, and elastic fibers and dilatation of the alveolar capillaries with no apparent epithelial covering. Other illustrations, presented by these authors, show thickened and relatively avascular septa covered by a cuboidal layer of cells. The pathogenesis of the lung changes in mitral stenosis has not been completely elucidated. The cuboidal cell membrane which lines the so-called alveoli may be derived from the peripheral growth of epithelial cells of the terminal bronchioles secondary to the fibrotic thickening of the septa with consequent loss of respiratory function.

It is also well known that the pulmonary parenchyma may undergo extensive histological alteration during the course of bacterial and virus pneumonia (Robertson and Uhley, '36; Loosli, '42, '49). Some areas of affected pneumonic lungs fail to return to a normal functioning state but instead undergo organization, atelectasis, and fibrosis. In such healed areas, one can usually demonstrate thick-walled avascular air spaces lined by cuboidal epithelial cells. The origin of this cellular membrane in the lungs of man has not been clearly determined. It is obvious that these epithelial-lined spaces in many areas do not represent alveoli, the normal architecture of the parenchyma having been completely altered by the pathologic process. This has been shown to be the case in experimental pulmonary influenza infection in mice (Loosli, '49). In this study, it was clearly evident that the non-functioning air spaces in the residual lesions resulted from the peripheral and hyperplastic growth of regenerating

bronchial epithelial cells into the atelectatic lung parenchyma. These influenza lung lesions resemble closely those (figs. 55-59, pp. 70-72) presented by Miller ('47). As in the case of the influenza lesions in the mouse, the air spaces which Miller describes in the lungs of man in all probability do not represent true alveoli, but healed non-functioning cyst-like spaces in a residual pathologic area.

Miller ('23, '25) emphasized the role of edema fluid in producing a swelling and separation of an epithelial membrane from the alveolar-wall surfaces. Likewise, he maintained that the pores of Kohn were not normal structures, but that the fluid exudate forced the shedding or separation of an epithelial membrane from opposing surfaces of an alveolar septum. In our study, marked pulmonary edema was produced in mice, rats, and dogs, but no epithelial membrane, or even a suggestion of a membrane similar to that described by Miller, was seen "lifted" from the alveolar surfaces. The alveolar pores (Macklin, '36b; Loosli, '37) were readily demonstrated in the edematous lungs by fibrin strands which extended through them into adjacent alveoli.

The situation was different in the lungs of frogs, where a thin plate-like membrane covers the opposing surfaces of an alveolar septum (Loosli, '38). In the ANTU treated frogs, this epithelial lining was separated as vesicles and sheets from the underlying capillary surfaces. This observation indicates that the method was, in all probability, suitable for separating similar membranes and cytoplasmic processes from the surfaces of the alveoli in the lungs of normal mice, rats, and dogs, if such existed. In the frog lungs, the thin cytoplasmic extensions of the epithelial cells whose nuclei were situated in the intercapillary spaces were clearly seen. No such picture was present in the mammalian lungs. The septal cells (the isolated epithelial cells of older authors) were not detached from the alveolar surfaces and no "flanges" or films of cytoplasm of these cells were seen lifted from the adjacent capillary loops. Likewise, non-nucleated

placques of Koelliker (1881) were not observed. Macklin ('47a) reported the presence of non-nucleated plaques in "chopped up" fresh lungs of mice. Later in 1947 and again in 1948, however, he withdrew his original observation and considered that the non-nucleated plaques which he had described were "smudges" of nucleated cells of diverse origin.

Some investigators still support the view of Miller (Bensley and Bensley, '35; Macklin, '36a). The majority, however, conclude that the respiratory portion of the mammalian lung does not possess an alveolar lining of endodermal origin. The various views on this subject have already been summarized (Loosli, '35, '37, '38). Macklin ('46) refers to the septal cells as epicytes and considers them to be residual epithelial cells. Others consider them mesenchymal in origin. Whatever the origin of the septal cells, the majority of recent investigators who have studied the pre- and post-natal development of the mammalian lungs consider them to be isolated cells (Palmer, '36; Dubreuil, Lacoste, and Raymond, '36; Barnard and Day, '37; Loosli, '38; Clements, '38; Ham and Baldwin, '41; Macklin, '46; Loosli in Maximow and Bloom, *Textbook of Histology*, '48).

In a study of the reaction of the septal cells in experimental pneumonia in the dog and monkey, Loosli ('42) considers them most likely to be fibroblasts of the alveolar wall which elaborate the reticular and elastic fibers and the ground substance that encloses the capillaries to form the alveolar covering. In this study, the septal cells, contrary to most observers, were rarely seen to desquamate and to give rise to the free macrophages in the pneumonic exudate.

It is our view that, until methods are developed which can demonstrate histologically a continuous membrane of endodermal origin covering the alveolar walls in normal mammalian lungs, including man, the presence of such a membrane must be denied.

SUMMARY AND CONCLUSIONS

1. Acute intoxication by intraperitoneal injection of ANTU resulted in marked pulmonary edema in frogs, mice, rats, and dogs.

2. In the frog lung, which possesses a histologically demonstrable epithelial membrane, the edema fluid lifted this flattened membrane from the underlying capillaries.

3. Although the lungs of ANTU treated mice, rats, and dogs showed extensive perivascular edema, lymphatic congestion, pleural effusion and alveolar edema, no membrane was lifted from the alveolar walls, and the septal cells appeared as isolated cells in intimate association with the connective tissue stroma. The cytoplasm of these cells was not seen to cover or to be lifted from the capillaries by the edema fluid. Non-nucleated plates were not seen.

4. Pores of Kohn were clearly demonstrated by fibrin strands in the exudate in the lungs of ANTU treated mice, rats, and dogs.

5. On the basis of this study, the presence of a continuous membrane of nucleated cells or non-nucleated plaques lining the alveoli of mice, rats, and dogs must be denied.

6. The origin of the isolated septal cells was not determined but their intimate association with the connective tissue stroma of the alveolar walls strongly points to their mesenchymal origin.

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PLATE 1

EXPLANATION OF FIGURES

1 Low power photomicrograph of an alveolus (al) in a frog's lung fixed by intravascular perfusion with a 10% neutral formalin solution. The blood vessels (v) and capillaries are empty due to the method of fixation. The alveolar septa (sep) are wide and covered by a double network of capillaries, except that portion lying adjacent to the pleura (pl). Hematoxylin-eosin-azure II stain. $\times 140$.

2 High power photomicrograph near a vessel (v) of part of a wall of an alveolus of frog's lung fixed as above. Note the termination of the cuboidal ciliated epithelial cells (cep). The epithelial membrane over the capillaries (c) is thin and blends with the cytoplasm of the epithelial cells (ep) in the intercapillary spaces. The nuclei of the capillary endothelial cells (end) appear elliptical in thin sections. Hematoxylin-eosin-azure II. $\times 880$.

3 High power photomicrograph of the alveolar wall lying adjacent to a blood vessel (v) in a frog's lung. Nucleated red blood cells are seen in the capillaries (c). Note the vacuolation of the cytoplasm of the epithelial cell (ep) which blends with the membrane covering the capillary. Hematoxylin-eosin-azure II. $\times 880$.

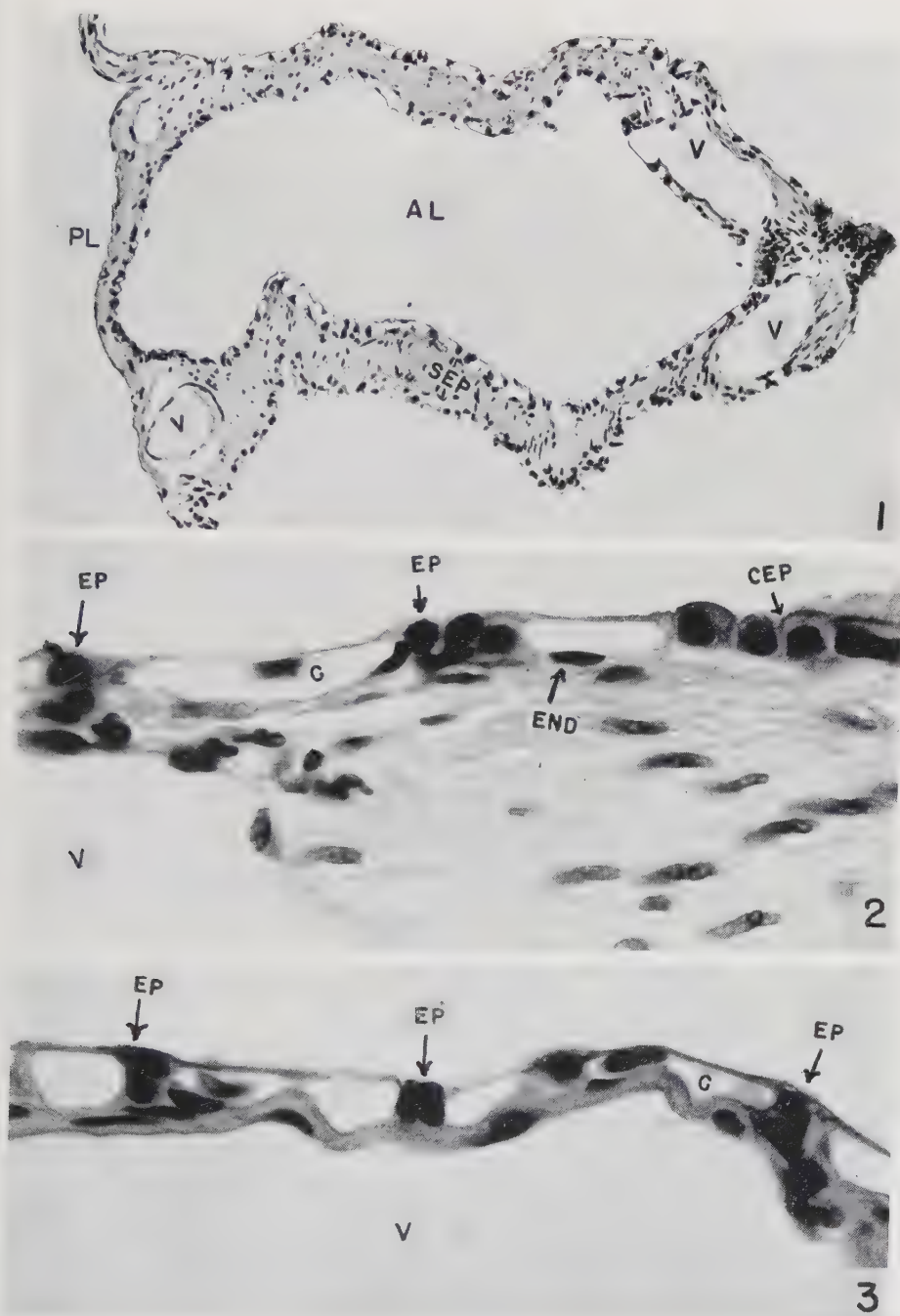


PLATE 2

EXPLANATION OF FIGURES

4 Medium high power photomicrograph of wall about the mouth of the air space in the lung of a frog killed 24 hours after an intraperitoneal injection of ANTU (8 gm per kilogram of body weight). Both the ciliated (cep) and flattened epithelial cells (ep) near the mouth of the air space have been separated as a membrane. Serous precipitate (sp) is present both outside and under the separated membrane. The connective tissue and smooth muscle cells of the septum (sep) are also swollen by edema fluid. Hematoxylin-eosin-azure II stain. $\times 350$.

5 A high power photomicrograph of the lung of a frog killed 27 hours after an intraperitoneal injection of ANTU (8 gm per kilogram of body weight). The epithelial covering (ep) has been lifted off the surface of the septum leaving the capillary endothelial cell (end) surface intact. Large nucleated red blood cells are seen in the capillaries (c). The fibers of the septum (sep) are separated by the edema fluid. Hematoxylin-eosin-azure II stain. $\times 880$.

6 A high power photomicrograph of a septum of the lung of a frog killed as described under figure 5. The epithelial cells (ep) with their cytoplasmic expansions have been separated from the opposing surfaces of the septum (sep). The nuclei of the epithelial cells (ep) are shown separated from their characteristic position in the intercapillary spaces. The capillary (c) endothelial cell (end) membrane remains intact. Hematoxylin-eosin-azure II stain. $\times 880$.

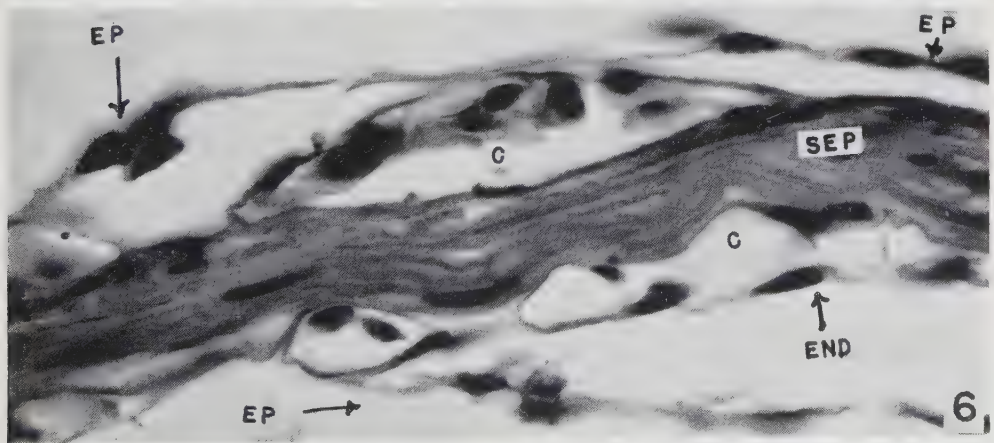
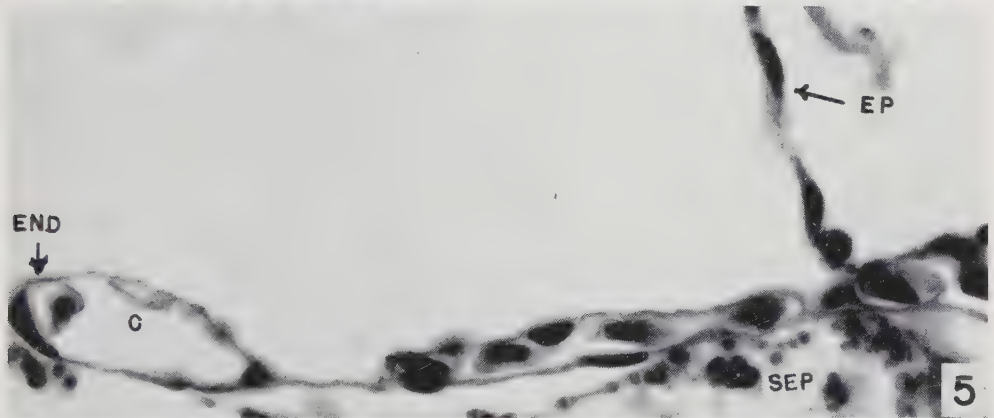
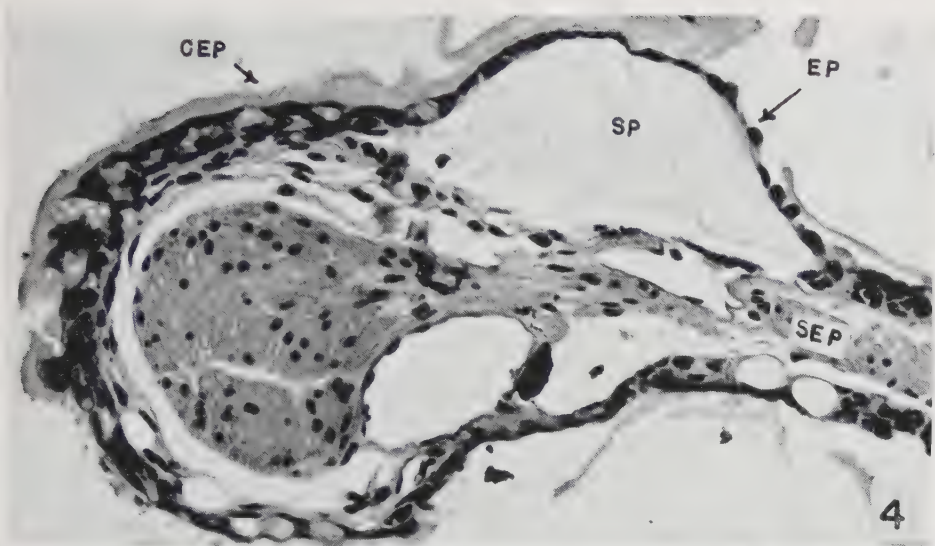


PLATE 3

EXPLANATION OF FIGURES

7 Low power photomicrograph of lung of a normal rat fixed intratracheally with Zenker-formol solution after the pulmonary vessels had been tied to keep the blood vessels (v) and capillaries filled with blood. The pleura (pl) is thin and the alveoli (al) are not completely expanded, whereas the terminal bronchiole (tbr) and alveolar ducts (ald) appear to be. Hematoxylin and orcein elastic-fiber stain. $\times 92$.

8 High power photomicrograph of the rat lung shown in figure 7, taken at the junction of the terminal bronchiole and alveolar duct. The epithelial cell (ep) membrane of the bronchiole terminates abruptly and is replaced by a single network of capillaries (c) which are filled with pale staining red blood cells. The elastic fibers (elf) support the capillaries. Note the thinness of the capillary membrane (cm) separating the capillary lumen from the alveolar space (al). Hematoxylin and orcein elastic-fiber stain. $\times 1025$.

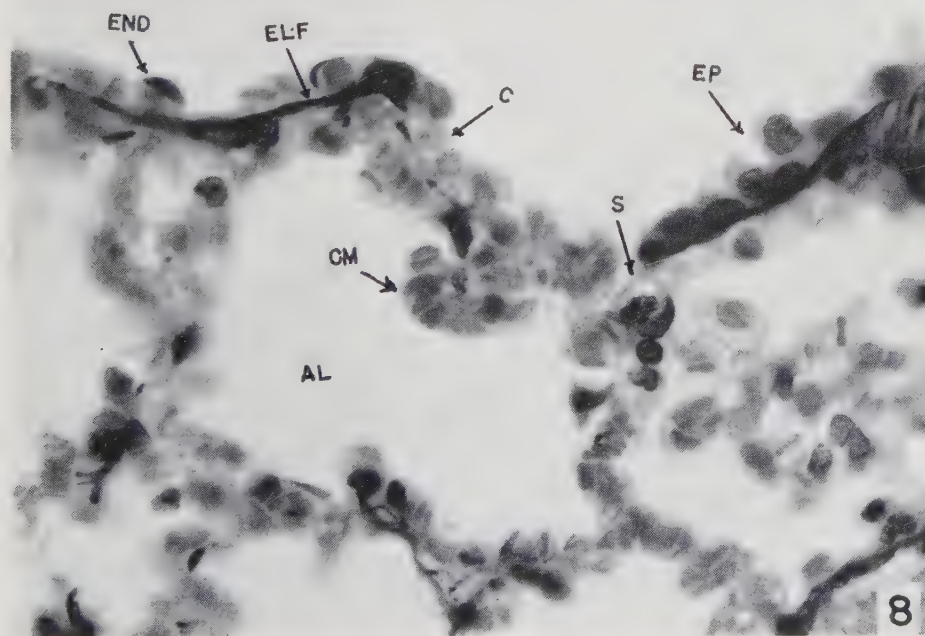


PLATE 4

EXPLANATION OF FIGURES

9 Low power photomicrograph of lung of rat killed $7\frac{1}{2}$ hours after the intraperitoneal injection of ANTU (50 mg per kilogram of body weight). The lungs were fixed as described under figure 7. There is edema of the vessel walls (v) and edematous precipitate (ed) in the alveolar spaces. There is no edema of the walls of the bronchioles (br). Hematoxylin-eosin-azure II stain. $\times 50$.

10 High power photomicrograph of the lung of the rat shown in figure 9. The alveoli contain abundant edematous and fibrinous exudate and a few cells (free pigment-filled macrophages [mac] and hematogenous lymphocytes, monocytes, and polymorphonuclear leucocytes). The fibrin strands appear to extend through the alveolar walls at points along the septa. The septal cells (s) have not become detached nor has a membrane been lifted from the capillary surfaces as is the case in the frog lung (compare with figs. 5 and 6). Hematoxylin-eosin-azure II stain. $\times 920$.

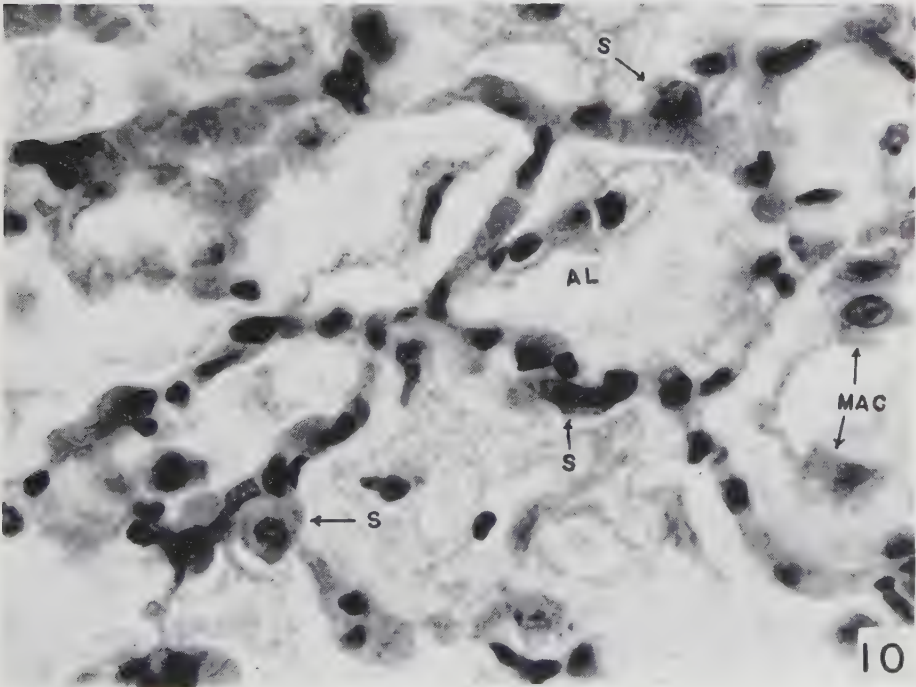
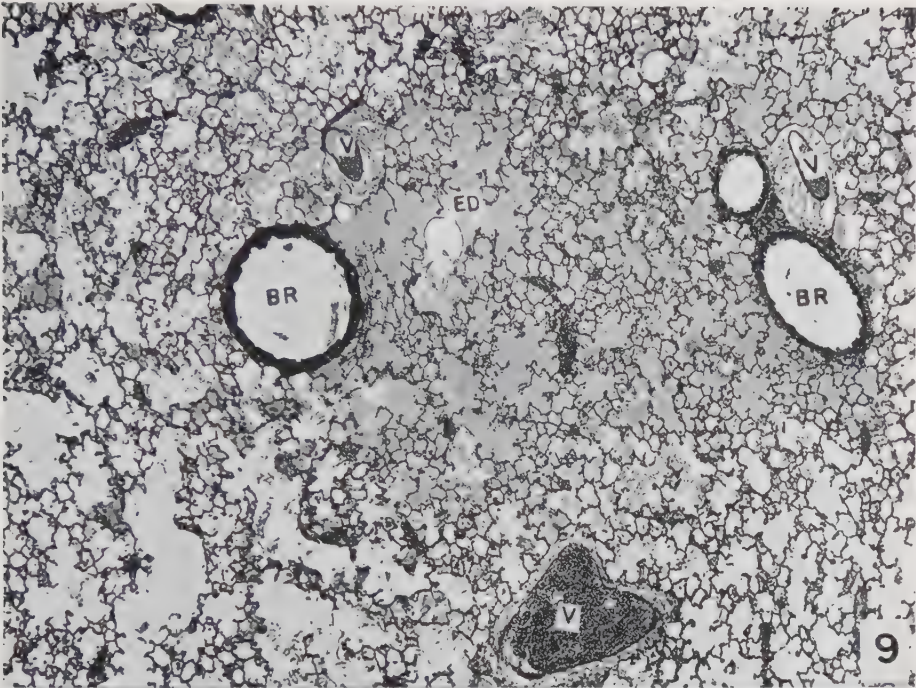


PLATE 5

EXPLANATION OF FIGURES

11 Low power photomicrograph of a lung of a mouse killed $11\frac{1}{2}$ hours after an intraperitoneal injection of ANTU (100 mg per kilogram of body weight). Edematous exudate (ed) is present throughout the alveolar spaces and extends into the lumina of the bronchioles (br). The vessel walls (v) are edematous but the bronchial walls are not. Hematoxylin-eosin-azure II stain. $\times 95$.

12 High power photomicrograph of a mouse lung shown in figure 11. The exudate consists essentially of serous precipitate containing fibrin strands which can be seen extending from one alveolus (al) to another through pores (p). The septal cells (s) have not been detached from the alveolar walls. Hematoxylin-eosin-azure II stain. $\times 920$.

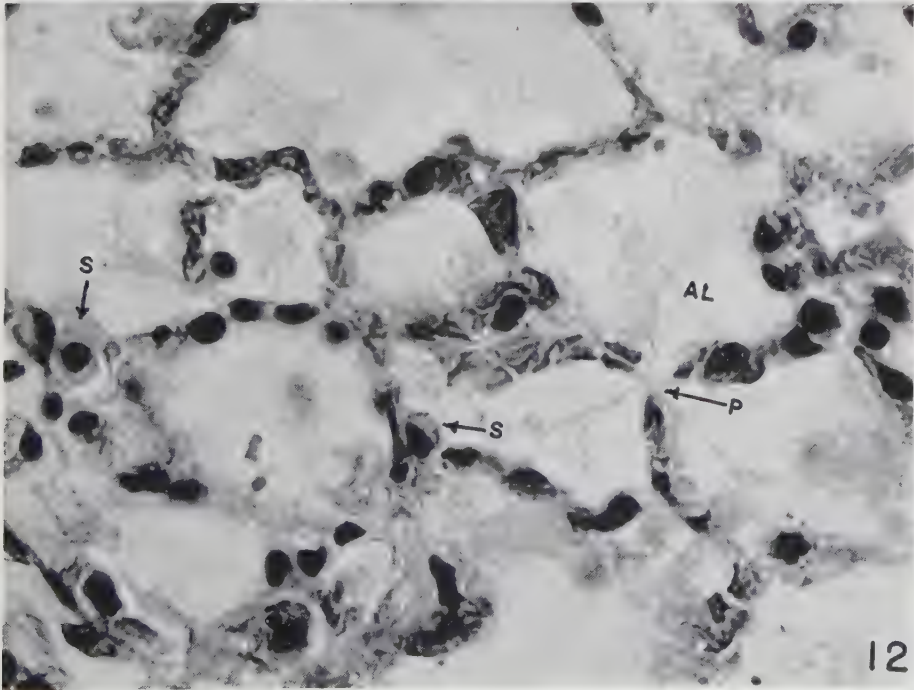
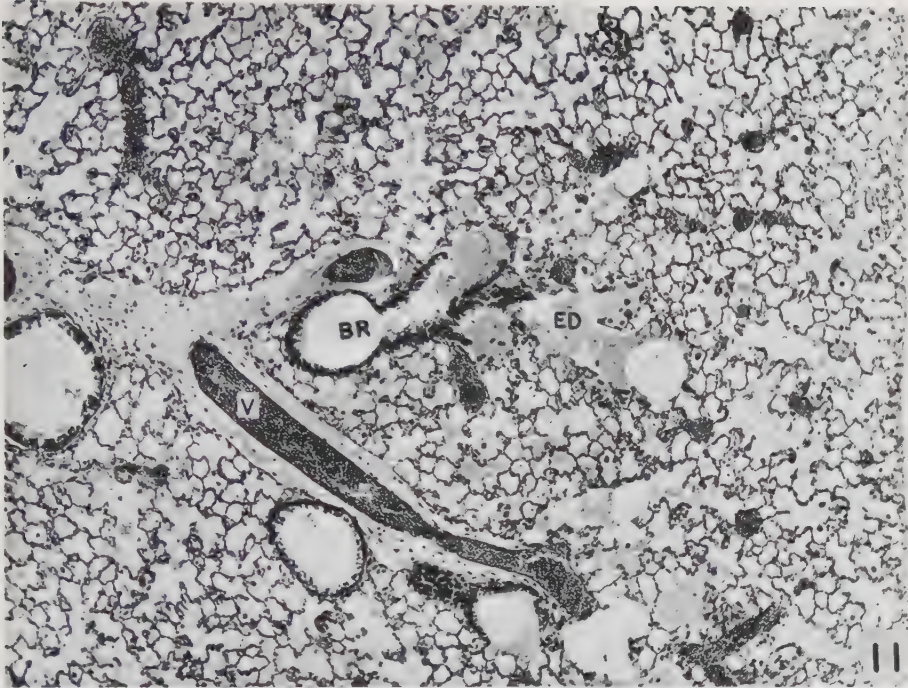
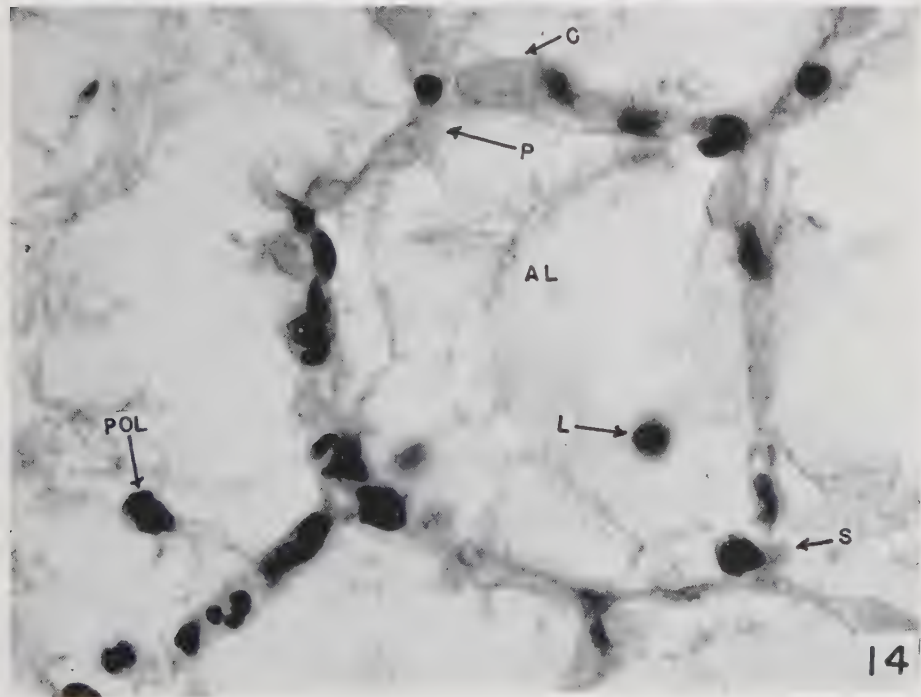
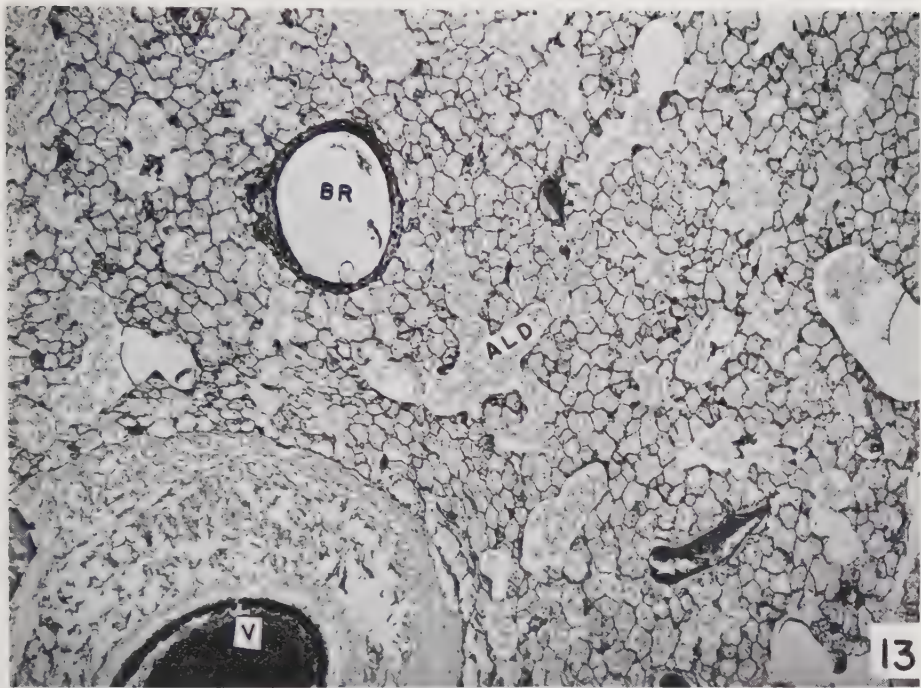


PLATE 6

EXPLANATION OF FIGURES

13 Low power photomicrograph of the lung of a dog sacrificed 23 hours after intraperitoneal injection of ANTU (10 mg per kilogram of body weight). The alveolar ducts (ald) and alveoli are filled with serous precipitate and fibrin. There is marked edema of the wall of the blood vessel (v) but the wall of the bronchiole appears to be free of edema. Hematoxylin-eosin-azure II. $\times 50$.

14 High power photomicrograph of a dog lung shown in figure 13. The fibrin strands pass through pores (p) from one alveolus (al) to another. The septal cells (s) are not detached. The cytoplasm of the septal cell (s) is seen to extend across the septum. The membrane over the capillaries (c) is thin and smooth and similar to that seen in the lung of the normal rat (fig. 8) and dog. A few cells of hematogenous origin (polymorphonuclear leucocytes [pol] and lymphocytes [l]) are present in the exudate. Hematoxylin-eosin-azure II stain. $\times 1025$.



LIMB REGENERATION IN *TRITURUS VIRIDESCENS* AS AFFECTED BY LETHAL DOSES OF RADIOACTIVE PHOSPHORUS

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FOUR FIGURES

INTRODUCTION

The effects of radiations on living tissues have for many years been of interest to the biologist. With the advent of nuclear fission and the subjection of many living forms to the emissions of radioactive isotopes, the need for further and more complete knowledge of radiation effects has become increasingly important. The radioactive isotope of phosphorus, phosphorus 32, has come to be used in numerous tracer studies. Some information has been published concerning its pathological effects but, as yet, neither its effects nor those of any other beta ray emitter on regenerating tissues have been described.

This paper describes in some detail and adds to observations previously presented in abstract form (Dent, '49) concerning the effects of intraperitoneal injections of relatively large doses of phosphorus 32 on stumps of amputated limbs in *Triturus viridescens*. The effects of sublethal doses are to be examined and it is anticipated that the findings obtained will appear in a subsequent publication.

MATERIALS AND METHODS

The animals used in this investigation were all fully mature adult males of *Triturus viridescens viridescens* (Rafinesque)

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which had been collected locally in Western Pennsylvania. It was found convenient to store the animals at $5^{\circ} \pm 1^{\circ}\text{C.}$ for indefinite periods of time after collection and prior to experimentation. At that temperature they were relatively immobile and did not require feeding. During the week preceding each experiment the animals to be used were removed from refrigeration and were shifted to containers at room temperature (19° – 23°C.) where they remained throughout the experimental period. While at room temperature the newts were each fed one pellet (about 0.1 gm) of lean ground beef per day. Individuals which did not eat readily were eliminated before experimentation began but during experimentation rejection of food was common among the experimental animals.

The phosphorus 32 used was obtained through the kind cooperation of the staff of the University of Pittsburgh Radiation Laboratory. It was received in aqueous solution in the form of H_3PO_4 . To the original solution NaOH was added in sufficient amount to neutralize the acid.

From time to time over a period of 4 months specimens of *Triturus* were injected intraperitoneally with phosphorus 32. The volume of solution injected (0.1–0.5 ml) was in each instance adjusted to produce one of three different radiation intensities in the animal. Thus, three groups of experimental animals were obtained: group A, 15 individuals, having received 166 microcuries per gram of body weight; group B, 12 individuals, 66 microcuries per gram of body weight; and group C, 16 animals, 33 microcuries per gram of body weight. The actual mass of phosphorus injected was not known but the total solids present in the radioactive solutions varied between some quantity less than 0.01 mg/ml and 2.3 mg/ml. Along with each experimental animal one control animal was injected with 0.5 ml of an aqueous solution of Na_2HPO_4 containing 6.0 mg Na_2HPO_4 per milliliter of water. During the period following injection the animals were kept, 4 to a container, in refrigerator jars (all animals in any one jar having received the same type of injection) about one-third full of

spring water. The water was changed the day following injection and thereafter at intervals of 5 to 6 days.

From both experimental and control animals the right forelimbs were amputated in the carpal region approximately one week after injection. Twenty-three days following amputation the limb stubs of several animals from each group were reamputated at the elbow joint. The tissue thus removed was fixed in alcoholic Bouin's solution (Galigher, '34) sectioned at 10 μ and stained with haemotoxylin and eosin-azure (Maximow, '24).

OBSERVATIONS

With the exception of three animals in group C, all experimentals were dead within a period of 40 days following

TABLE 1

Relation between dosage and mortality in specimens of Triturus viridescens injected with aqueous solutions containing phosphorus 32

GROUP	NO. INDIVIDUALS IN GROUP	AMT. P ³² INJECTED (In μ c/gm body wt.)	AV. NO. DAYS ALIVE AFTER INJ.	NO. DAYS BEFORE FIRST DEATH IN GROUP	NO. DAYS BEFORE LAST DEATH IN GROUP
A	15	166	25	16	33
B	12	66	30	22	32
C	16	33	36	22	83
Controls	43	Injected with 0.5 ml 0.042 M Na ₂ HPO ₄	4 died in course of investigation		

injection (see table 1). With few exceptions, the animals of group A ceased to accept food during the first week injections were made. Rejection of food was general in group B after two weeks and in group C three weeks following injection. In many instances, particularly in groups A and B, just prior to death external lesions were observed in the sub-lingual region. The cause of the deaths among the control animals is not known. The other control animals were quite active and apparently healthy for the two weeks during which they

were observed following the demise of their companion experimental individuals.

Although no quantitative tests were made, the water in which the animals were put after injection gave relatively high emission counts on the following day indicating a rapid release of phosphorus.

Among the control animals well-formed blastemata were usually visible externally after about 3 to 4 weeks following amputation and beginning digit formation could be seen after 5 to 7 weeks. No external indication of blastema formation was seen among the experimental animals except in group C. In that group mounds of tissue resembling blastemata developed on the limb stumps of the three exceptional individuals which lived longer than 40 days following injection (45, 61, and 83 days; respectively) but digit formation was not observed.

Longitudinal sections of limbs reamputated and fixed 23 days after amputation were examined. Figure 1 is a photomicrograph of a typical median longitudinal section of a limb from a control animal. The control limbs at this stage of regeneration exhibited the following characteristics: The wound epithelium was relatively thick, being made up internally of several layers of closely packed cells with nuclei of almost uniform size and shape and externally of a layer of flattened squamous cells. Beneath the wound epithelium free regeneration cells were gathered in large numbers. Mitotic figures were found in both the epithelium and sub-epithelium with average incidence of about 5 per median section.

In limbs from animals of group A undifferentiated cells were apparently entirely absent beneath the epithelium which covered the cut surface. The epithelium itself was relatively thin but was made up for the most part of cells with greatly enlarged and lobate nuclei (fig. 2). Vascularization was frequently abundant (fig. 2). Mitotic figures were seen rarely, roughly one in each 20 sections studied.

In general, no dissimilarity between limbs from animals of groups B and C was noted. Wound epithelia covering the

amputation surfaces, as a rule, showed considerable thickening. Also, many of the nuclei of these layers were enlarged and lobate (fig. 3). In a few instances when enlarged cells were not present in the epithelium it was made up of flattened cells, as if the deep epithelial layers were absent and the superficial layer were expanded (fig. 4) giving the general appearance of scar tissue. Some massing of regeneration-type cells was evident in the sub-epithelial region; however, it will be noted from examination of figures 3 and 4 that the cell density per unit volume of tissue (Butler, '35) is apparently much less than in the limb tips from control animals and that there is a tendency for these tissues to be loose and fibrous. Although mitotic figures were seen, their incidence was quite low (approximately one figure per each three median sections studied).

The observations described in the preceding paragraphs apply to limbs sectioned 23 days following amputation. It was mentioned earlier that three individuals from group C lived considerably longer than the other experimental animals and that blastema formation appeared to take place on the limb stumps of those animals. After reamputation the limbs of two of these animals were sectioned. The conical tip of one merely resembled a blastema externally but was internally in about the same condition as the limb tips of the 23-day-old amputations of group C. The other one showed internal clumpings of cells indicating beginning digit formation.

DISCUSSION

The results cited in table 1 would appear to establish the dosages of phosphorus 32 used in this investigation as lethal. Further, they give some indication that the average length of time which the newts lived after injection is inversely correlated with the activity of the phosphorus injected. It might be suggested that the observed mortalities resulted from the presence of some unidentified toxic agent in the radioactive solutions; however, the solutions were made up from several different samples of phosphorus 32 each having

a different activity per unit volume when received. Thus, some of the material which was actually injected was as much as 5 times as dilute as other material of the same activity strength. The observed mortality was correlated with the activity per unit volume of the injected material rather than with the degree of dilution per unit volume.

Whether or not regeneration would have been completed in any of the experimental animals is obviously a question which cannot be answered with the data at hand; however, in the experimental animals the tissues at the sites of amputation differ markedly from the typical condition. Mitosis which is, of course, essential to regeneration is almost halted by the strongest dosage used (166 microcuries/gm) and greatly depressed in the weaker dosages.

The massed cells which make up the blastemata of the controls are absent in group A and scarce in groups B and C. Rose ('48) has produced evidence to show that the cells of the early blastema come from the thickened wound epithelium. The epithelium covering the limb stumps of the animals of group A bears almost no resemblance and that covering limb stumps of groups B and C little resemblance to the epithelium of the control limbs with its several layers of uniformly shaped cells. It would seem unlikely that the altered epithelium of the experimental limbs would make a typical contribution to forming blastemata. Both Butler ('35) and Horn ('42) who studied, respectively, the effects of X-rays and neutrons on regenerating limbs of *Amblystoma* larvae observed that the non-regenerating blastemata of irradiated limbs were low in cell density per unit volume of tissue. The lack of blastemata or the slowing down of blastema formation probably results directly from a general inhibition of mitosis and indirectly, possibly, from distortion of the epithelial cells by radiation effects.

SUMMARY

1. Adult specimens of *Triturus viridescens* were injected intraperitoneally with solutions containing phosphorus 32 in

doses which produced radiation intensities of 33, 66, or 166 microcuries per gram of body weight at the time of injection. Right forelimbs were excised in the carpal region approximately one week following injection.

2. All three dosages were apparently lethal since all the experimental animals died.

3. Histological examination of limb stumps removed 23 days following the original excision revealed that:

(a) Typical blastemata were developing on limb stumps of control animals.

(b) Limb stumps from experimental animals which had received 66 or 33 microcuries per gram gave some indication of beginning regeneration; however, the blastema region was characterized by:

1. Atypical epithelium which either contained many enlarged and lobate cells or was made up entirely of flattened cells of the squamous type.

2. A relative scarcity of mitotic figures.

3. A relatively low number of cells per unit volume of tissue.

(c) Epithelium covering limb stumps removed from animals which had been treated with 166 microcuries per gram of body weight was made up, for the most part, of enlarged and distorted cells. No indication of blastema formation was seen in the sub-epithelial region. Mitotic figures were extremely rare.

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PLATE 1

EXPLANATION OF FIGURES

1 Median section of blastema from control limb fixed 23 days after original excision. Note thick epithelium composed of uniformly shaped cells and numerous undifferentiated cells in the sub-epithelial region.

2 Median section of limb stump from animal injected with 166 microcuries of phosphorus 32/gm of body weight. Tissues fixed 23 days after amputation in the carpal region. Beneath the thin squamousal layer is a heavily vascularized region which is separated from the differentiated tissues of the limb stump by a layer of epithelial cells which runs across the photograph horizontally and divides it roughly in half. Note also absence of undifferentiated regeneration cells.

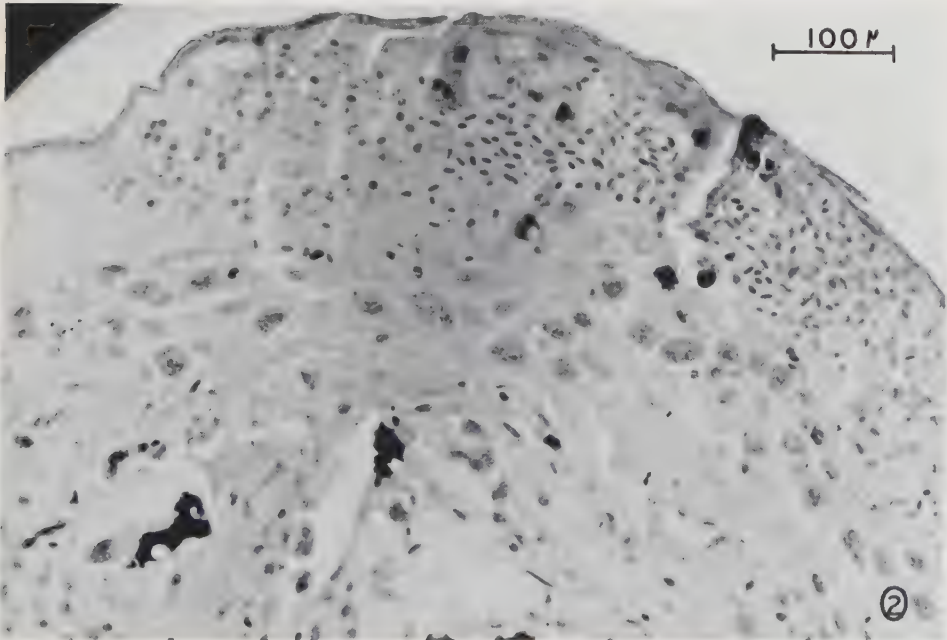
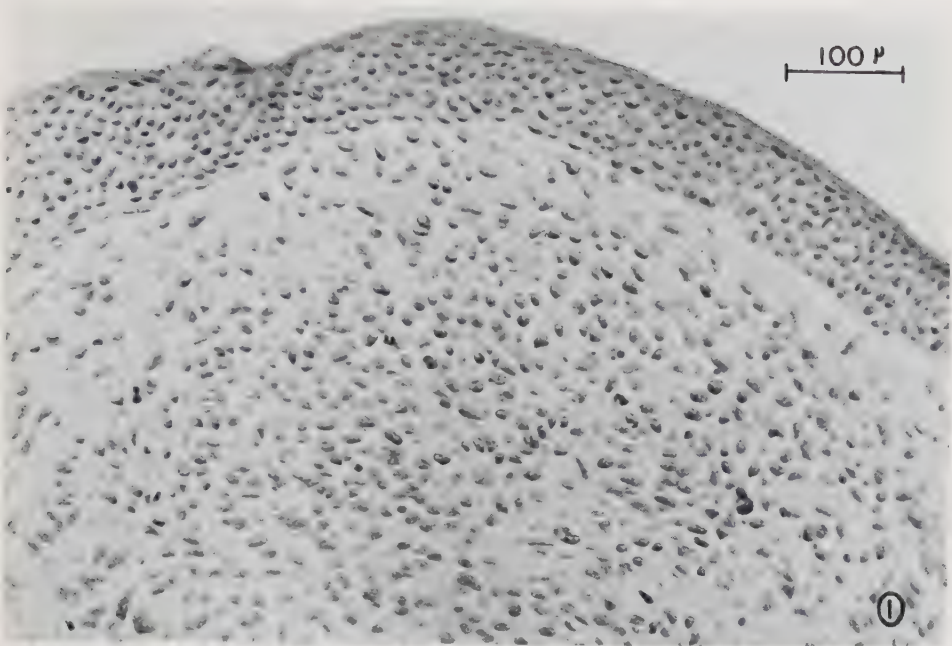
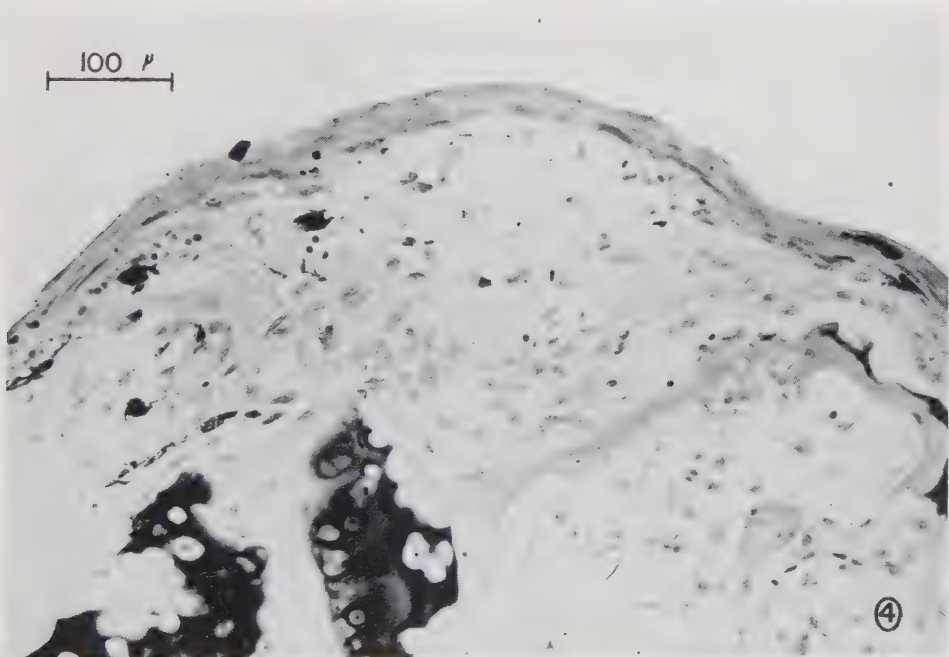
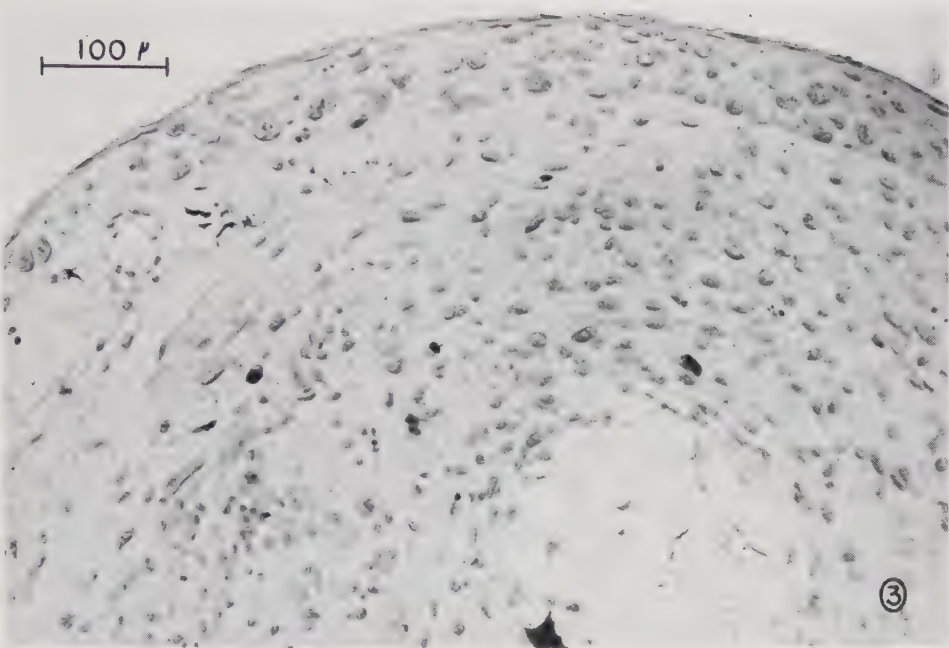


PLATE 2

EXPLANATION OF FIGURES

3 and 4 Tips of limb stumps fixed 23 days after original excision. Animals injected with 66 microcuries of phosphorus 32 per gram of body weight. The epithelium in figure 3 is relatively thick, but is composed primarily of loosely distributed cells with irregularly shaped nuclei. In figure 4 epithelium is compact but its cells are elongated tangentially with respect to the external surface of the stump. In both sections there is a pronounced lack of undifferentiated sub-epithelial tissue.



THE EFFECT OF TESTOSTERONE PROPIONATE ON THE CONNECTIVE TISSUE OF THE HEAD APPENDICES AND THE SKIN OF THE CAPON

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ELEVEN FIGURES

INTRODUCTION

It is a well known fact that the connective tissue of certain skin formations of the head and neck regions of birds are under the direct control of androgens. Virchow (1851) mentioned the mucoid character of the connective tissue of the cock's comb. Histological investigations of more recent date, especially those of Champy and his collaborators ('25, '26, '29), Hardesty ('31), and Garrault ('34) have proved that in the normal cock's comb there is an intermediate layer of characteristic mucoid connective tissue which depends on the presence of the male hormone. Heringa and Weidinger ('40) have shown that the mucoid substance of this tissue is responsible for its typically high water-binding capacity. Heringa ('48) has pointed out that the presence of the mucoid ground substance is also related to structural changes in the connective tissue fibrils, the connective tissue of the capon's comb containing predominantly dense collagenous fibers while that of the normal cock consists mainly of reticular fibrils.

It is probable, however, that the sex hormones also influence the remaining skin and connective tissue. Some obser-

¹The author wishes to thank Professor Dr. G. C. Heringa for suggesting this work and for the help he has given to him.

vations indicate that this action affects the ground substance of the connective tissue. For example, the increase in water content of the skin after treatment with sex hormones (Taylor and Sprunt, '43) could be explained by an increase of the water-binding mucoid ground substance. The difference in the connective tissue permeability under the influence of sex hormones (Sprunt, McDearman and Raper, '38; Lurie and Zappadosi, '39), seems to be a function of the mucoid ground substance also, and is another indication of this mode of action. On the other hand, Herrick ('45) has indicated that testosterone propionate causes an increase of collagenous fibers in the skin of capons. Collagen formation probably also plays a role in the production of fibroids after estrogen treatment (Lacassagne, '35; Lipschütz et al., '40, '41).

STATEMENT OF PROBLEM

The above facts led us, first, to the problem of the specific hormonal reaction which results in the development of a characteristic mucoid connective tissue in certain skin regions and, secondly, to whether the sex hormones have any effect on the remaining cutaneous connective tissue and, if so, the relation between these two reactions. In the present investigation special attention was given to the following problems:

1. The morphological changes in the fibrillar elements of the connective tissue of the capon's head appendices after experimental regeneration and following regression.
2. The development, properties and disappearance of the metachromatic (mucoid) ground substance in the connective tissue of these organs.
3. The possible occurrence of phosphatase activity in the head appendices of capons during regeneration and regression.
4. The characteristics of the growth of the comb, i.e., whether the increase in volume is due to physical swelling ("expansion growth" according to Heringa, '40) or to biological (cell) growth.

5. Finally, the possible changes in the structure of the cutaneous connective tissue under the influence of different sexual conditions.

MATERIAL AND TECHNIC

Animals and hormone. Fourteen white Leghorns were used. There were 10 capons, two years of age and castrated at 6 weeks, with an average weight of 2,800 gm and 4 control cocks, about two years of age with an average weight of 2,100 gm. Testosterone propionate ("Neohombreol," Organon²) was the androgenic hormone used. Daily intramuscular injections were given over a period of 10 days during February and March in individual doses of 1.0 mg.

Measurement of the comb's growth. The growth of the comb area was measured daily throughout the first 29 days of the experiment, according to the planimetric method introduced by Freud ('31).

General histological methods. The capons treated in this manner were killed on the 4th, 8th, 11th, 15th, 21st and 29th days of the experiment. An untreated capon and two normal cocks were killed during this period. Histological examination was made of the comb and wattle. The specimens were fixed in 80% alcohol, ether-formol (according to Leyns and Gaulhofer, '39), 4% basic lead acetate, formol 1:4, and Zenker's fluid. The material fixed in ether-formol was further treated according to the gelatine method of Heringa ('28), which proved to be of extraordinary value for the soft and easily shrinkable material. The thickness of the gelatine frozen sections was approximately 15 μ . The rest of the material was embedded as usual in paraffin and sectioned at 8 μ . For general purposes the following staining methods were used: Ehrlich's hematoxylin and erythrosin, Heidenhain's Azan and the nuclear method of Feulgen.

Investigation of the fibrillar compounds. Sections were stained with orcein, resorcin-fuchsin, reticulum impregna-

² Thanks are due the Biological Laboratories, N. V. Organon, Oss, Holland (Dr. M. Tausk, Director) for their gift of the hormone preparation and capons used in this work.

tion of Laguesse and with Mallory mixture. The optical properties of the fiber substance were examined in frozen sections under the polarizing microscope. To determine the swelling properties of both the fibrillar and ground substance, we used the swelling method worked out by Graubert ('48) in this laboratory. This method is based upon the antagonistic swelling properties of collagen and mucoid substance in the connective tissue. Connective tissue with much mucoid substance swells more in distilled water than in a salt solution, while in connective tissue containing much collagen, the contrary occurs, since the swelling of collagen is promoted by salt. Tissues rich in collagen show a very marked swelling in acid (pH 2.4) but do not swell to any great extent in the neutral media. On the contrary, tissues with much mucoid substance do not swell in the acid area, but exhibit a maximum of swelling in the neutral area (pH 7.0). We used distilled water and 0.9% sodium chloride for the experiments concerning the influence of the salt. In the experiments on the swelling at various pH's, we used the following buffers: for a pH from 2.4 to 6.0, potassium biphthalate—hydrochloric acid (or sodium hydroxide); for a pH of 6.0 to 8.0, the phosphate buffer of Sørensen. Before use, these solutions were diluted, 1:10, and a small amount of mercury iodide was added as preservative. The pieces of the tissue (comb, wattle, skin of the breast and back), each weighing about 50 mg, were weighed, and put in 50 ml of the solution for 24 hours. The pieces were then weighed again, and the increase in weight was expressed in percentages. Most of the values were based on the average of three to 6 specimens. Further, we studied the fiber substance of the comb using the X-ray spectrograph. For this investigation thin strips of the capon's and cock's comb were parallelized by stretching and dried; in some cases the mucoid substance was removed by treatment with hyaluronidase.³

³ Hyaluronidase from bull testes, prepared according to the method of Claude and Duran-Reynals ('37), was obtained through the kindness of Dr. h. c. E. Dingemans, Pharmaco-Therapeutic Laboratory of the University of Amsterdam.

Investigation of the mucoid substance. For this purpose, histological sections were stained with toluidin blue in 0.5% aqueous solution, mucicarmine and the Bauer polysaccharide method in different modifications. Some slides were treated with a 0.001% hyaluronidase in physiological saline solution at 37° for one to 24 hours and they were then stained with toluidin blue. The hyaluronidase solution, which acted upon the sections, was thereafter tested for reducing substances using the Molisch reaction.

Phosphatase estimation. We used the histochemical reaction of Gomori, modified by Ruyter and Neumann ('49) in this laboratory, for the localization of alkaline phosphatase in histological sections. In one case, chemical determination was made according to the method of Kroon, Neumann and Krayenhoff Slood ('44).

RESULTS

1. Growth curve

The results of the planimetric measurements of the comb area were expressed in percentages and the arithmetic mean was determined. When more than three values were available, the standard deviation was calculated. In figure 1, the regeneration of the capon's comb after treatment with hormone follows a typical S-shaped growth curve, while the regression is characterized by a parabola.

2. The connective tissue of the head appendices

(a) *The fibrillar compounds.* In the connective tissue of the intermediate layer of the capon's comb, we found compact, collagenous fibers. These extended from the subepithelial capillaries up to the central core. They often followed a wave-like course, did not anastomose, and formed a compact net of fibers lying close together (fig. 2A and fig. 3). They gave a dark, violet-black color with silver impregnation, but were also easily stained with collagen-staining dyes, e.g., aniline blue. We were able to demonstrate by polarizing

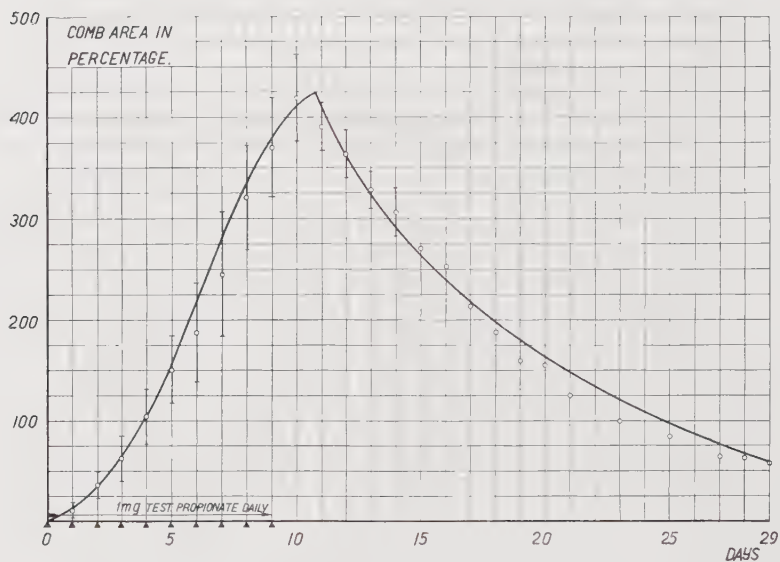


Fig. 1 Growth- and regression curve of the capon's comb after treatment with 10 mg testosterone propionate.

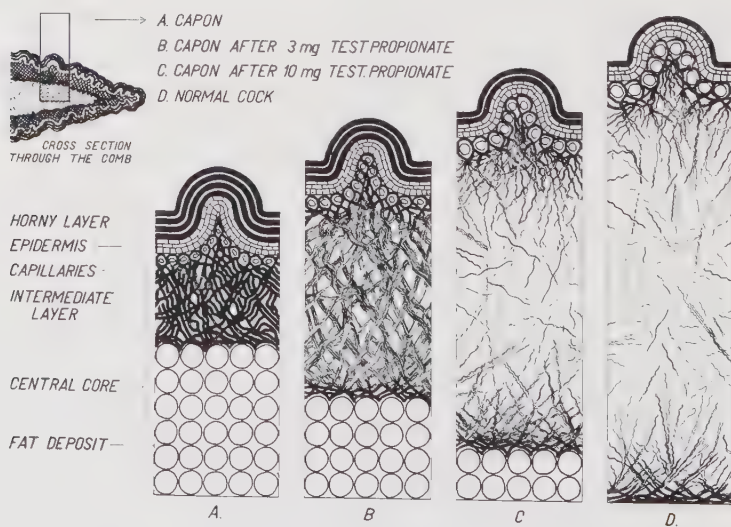


Fig. 2 Schematic demonstration of the effect of the male hormone on the connective tissue of the comb.

microscopy, that the fibrils of the intermediate layer of the capon's comb showed clear double refraction, while the basal membrane and the reticulum of the capillaries were optically clear. The swelling experiments demonstrated that no compound capable of marked swelling was present in the capon's comb (fig. 9 and table 1). The tissue of the capon's comb



Fig. 3 Comb of the untreated capon. Fixed in ether-formol, frozen section after gelatine embedding, silver impregnation of Laguesse. $\times 69$.

gave, however, an X-ray diagram corresponding to that of typical collagen. We also found a small number of elastic fibrils in the sections of the capon's comb which, together with fibers of the collagen-type, formed a pattern around the subepithelial capillaries.

After treatment with male hormone for three days we found that distinct changes in the connective tissue of the capon's comb were already present. These changes took a

definite course, beginning at the base of the comb and spreading upward to the points. The first remarkable occurrence was the change in the staining properties of this tissue. The clear, sharp borders of the fibers disappeared. The fibers were not as deeply stained with silver and rather poorly defined with Azan. This indefinite staining was seen particularly in



Fig. 4 Comb of the capon, treated with 3 mg testosterone propionate. Technic as in figure 3. $\times 69$.

the area surrounding the subepithelial capillaries. One could see that the fibers lost their compact, firm structure, grew more vague and seemed to break up into fine elementary fibrils (fig. 2B and fig. 4). In the next phase, after the 7th day of treatment, we found that the fibers were usually broken into fine fibrils and spread by the ground substance. They kept their wavy course and often presented a disorganized appearance. After the 10th day of treatment, one could see

that the whole area of the intermediate layer of the comb was filled with strongly metachromatic ground substance, while the fibers were clearly split into small elementary fibrils (fig. 2C and fig. 5). Under the polarizing microscope, these elementary fibrils showed definite double refraction, similar to the fibers of the untreated capon's comb. The elastic fibers did not change during hormone treatment. They did

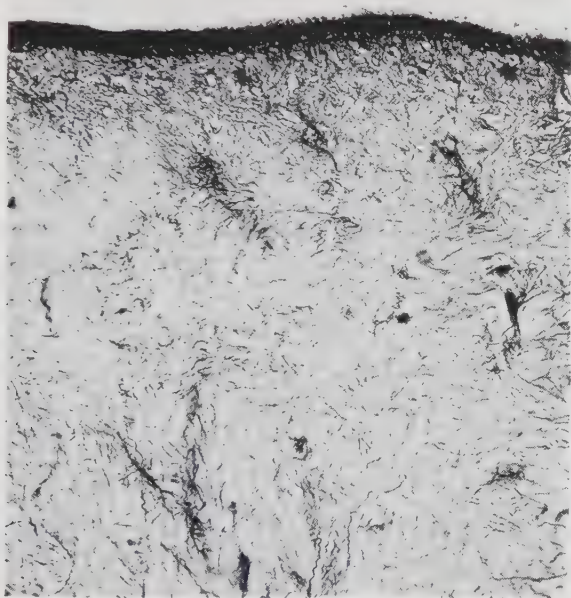


Fig. 5 Comb of the capon, treated with 10 mg testosterone propionate. Technic as in figure 3. $\times 69$.

not increase in number and their chief localization under the epithelium remained the same as in the untreated capon's comb.

In the intermediate layer of the normal cock's comb, thin connective tissue fibrils were found. They were laid in the amorphous mass of metachromatic ground substance. One could see that these fine fibrils, often having a wavy course, were separate portions of thicker bundles (fig. 2D and fig.

6). Their staining properties were almost identical with those of the fibrils of the combs of the injected capons. Following silver impregnation they had a violet color, but they also retained their ability to take up collagen stains. They showed double refraction under the polarizing microscope which was identical with that of the fibrils in combs of injected capons. The X-ray diagram of the connective tissue of the cock's



Fig. 6 Comb of the normal cock. Technic as in figure 3. $\times 140$.

comb (fig. 7) showed the typical spectrum of collagen, similar to the diagram of the capon's comb. In the cock's comb, the elastic fibrils occupied practically the same position as in the capon's comb.

After the cessation of hormone treatment, the histological changes in the capon's comb during the regression phase took place in the opposite direction from those occurring during the phase of regeneration. In other words, they were noted first at the points and later at the base. The thin fibrils came closer together, the ground substance disappeared and thick

fiber complexes were formed. On the 29th day of the experiment, the histological picture of the fiber substance of the comb was identical to that of the untreated capon.

The histological structure of the other head appendices, e.g., wattles, was for the most part identical to that of the comb. The fiber substance of the wattle showed the same picture as that of the comb and exhibited similar properties of swelling and double refraction. The changes of the wattles during the hormone treatment were identical with those of the comb.



Fig. 7 X-ray diagram of the fiber substance of the normal cock's comb.

(b) *The metachromatic substance.* We found no metachromatic substance in the connective tissue of the capon's comb using toluidin blue. Only single mast cells could be demonstrated beneath the epithelium. Mucicarmine also gave negative results, as did the Bauer polysaccharide reaction. Similarly, in the region of the first morphological changes after hormone treatment which we described as a loss of staining capacity and of firm fiber structure (fig. 2B and fig. 4), we still found no metachromasia and the number of mast cells remained unchanged. In the next phase, when fibers were

broken into fine fibrils, diffuse metachromasia appeared in the interstitial spaces. The metachromatic substance was localized around the destroyed fiber substance and often in the neighborhood of connective tissue cells. It had, after most fixations and after paraffin embedding, a fibrous, granulated appearance, but after ether-formol and gelatine embedding it had a more or less homogenous appearance. First the metachromasia was seen only in isolated spots. These extended as the process continued and gradually filled up the whole area of the intermediate layer. We noted that during this process no changes in the mast cells could be demonstrated.

The histological picture of the cock's comb was practically identical with that of a capon's comb after 7 to 10 days hormone treatment. Thin connective tissue fibrils were embedded in and separated from each other by strongly metachromatic ground substance.

The main phenomenon during the regression of the combs of previously treated capons was the disappearance of the metachromatic ground substance. It became fibrous and disappeared entirely when the fibrils came sufficiently close together. We frequently saw a thickening of the metachromatic ground substance along the fibrils which appeared to be glued together. In general, the whole process of regression was opposite to that of formation. It should be noted, however, that the course was much slower than that of formation. Comparing sections of equally large combs in the phases of formation and regression, we saw that the structure of the two was not identical.

The metachromatic ground substance had a swollen character and was very viscous. Its staining properties were the same as those of other mesodermal mucopolysaccharides. Very obvious metachromasia was obtained with toluidin blue. Staining with "mucin stain" such as mucicarmine was possible, but not very strong and less clear than that of mucins of epithelial origin. The polysaccharide reaction of Bauer, which we used in different modifications, was negative. After treatment of the sections of the cock's comb with

hyaluronidase, the metachromasia of the ground substance practically disappeared after one hour. On the other hand, the metachromasia of the vessel walls remained unchanged. The hyaluronidase solution, which had acted upon the sections of cock's comb, gave, thereafter, a positive Molisch reaction. Unfortunately, the swelling experiments with the Graubert's method gave no quantitative results as regards the mucoid substance. In all likelihood this was due to the fact that the mucoid substance from these tissues ran out and settled at the bottom of the vessel after a few hours, and so, on the whole or in part did not take part in the weight increase. This loss of weight greatly modified the swelling curves of comb and wattle (fig. 10 and fig. 11). Similarly,

TABLE 1

The weight increase, due to the swelling of fresh tissue in distilled water and in a 0.9% sodium chloride solution, is expressed in percentages

	COMB		WATTLE	
	Distilled water	Salt solution	Distilled water	Salt solution
Capon	29%	23%	67%	23%
Cock	48%	28%	55%	17%

the results of the salt-water swelling (table 1) did not reflect the remarkably great structural differences between the comb and wattle of capons and cocks. We saw, however, that the substance which settled at the bottom of the vessels had a great capacity for swelling.

(c) *The phosphatase activity.* With the histochemical reaction, we could not demonstrate any phosphatase activity in the connective tissue of the comb of either the capon or the normal cock. Chemical determination in the cock's comb gave values corresponding to 5 Bodansky Units for both the acid and alkaline phosphatase. On the other hand, we found a distinct positive reaction in the vessel walls during the phase of formation (after 4, 8 and 11 days) and in the phase of regression (15 and 21 days). In these phases most of the alka-

line phosphatase activity was located in the intima of the vessel walls, and a small amount also in the connective tissue cells around the blood vessels (fig. 8). We also could observe a marked vasodilatation during the formation phase, and vasoconstriction during the regression.

(d) *The connective tissue cells.* The connective tissue of the capon's comb, as is true of the connective tissue of poultry in general, is very rich in cells of the fibrocyte type. Lymphocytes may also be found either scattered diffusely or in small lymph nodes. During the course of hormone treatment,

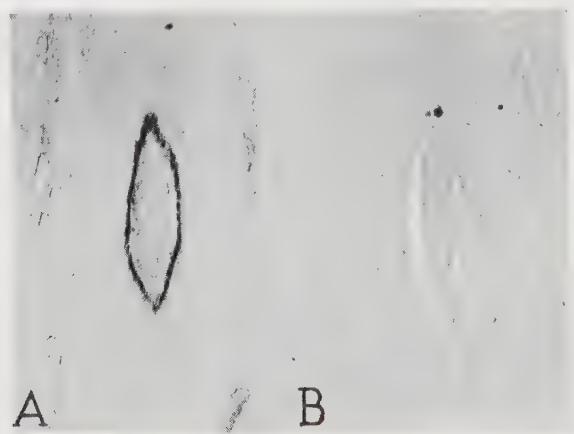


Fig. 8 Comb of the capon, treated with 7 mg testosterone propionate. A. Alkaline phosphatase demonstration in the vessel wall. B, control. $\times 125$.

when the metachromatic substance appeared, the connective tissue cells of the capon's comb changed in morphology. The distance between the fibers became larger, the cells were more visible, and took the stellar aspect of fibroblasts. In the cock's comb, the connective tissue cells of the intermediate layer showed a similar aspect. Although we saw definite changes in the morphological appearance of these cells, we could say almost certainly that there was no quantitative change. We could not find mitotic figures in the connective tissue of the capon's comb after hormone treatment. In all phases, it was typical to find that the more ground substance there was in

the connective tissue, the fewer cells there were in the same field. Hence we concluded that the number of the cells remained the same.

3. *The connective tissue of the skin of the body*

The swelling experiments on the pH range showed a quantitative difference between the swelling capacity of the capon's

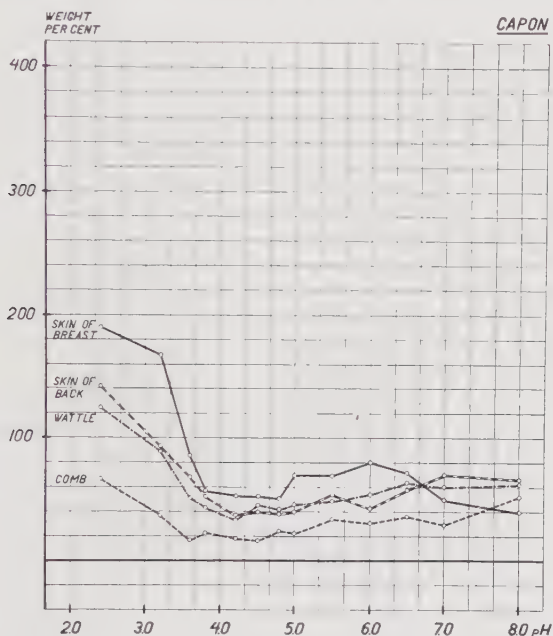


Fig. 9 Swelling curves of different skin regions of the untreated capon.

skin and cock's skin. According to Graubert ('48) this depends on a different behavior of the swelling of the respective fiber substances. Further, the experiments on swelling showed distinct differences between different skin areas of the same animal. This difference seems to be analogous to the difference between young and old skin according to Heringa and Weidinger ('42).

The swelling curves from untreated capons (fig. 9) showed approximately the same direction in their course. They all

had a common characteristic, namely, at the lower pH's they showed a certain amount of swelling. The swelling capacity was lowest in the comb and greatest in the skin of the breast, the skin of the back and the wattle lying in between. The range between the extremes at pH 2.4 was about 120%.

The curves from the treated capon (fig. 10) diverged from each other in the acid region. At a pH of 2.4, they reached

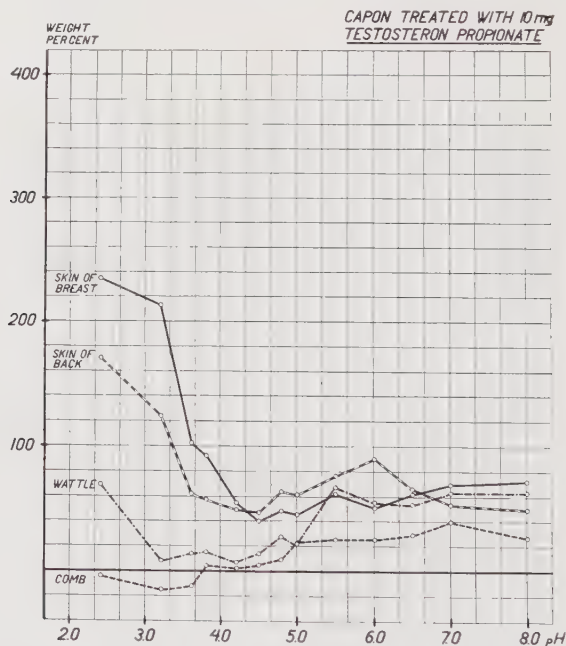


Fig. 10 Swelling curves of different skin regions of the capon, treated with 10 mg testosterone propionate.

an extent of about 250%. The curves of the comb and wattle had about the same course as those in the cock, those of the breast and back skin, however, differed to only a small degree from the corresponding curves of the untreated capon.

The swelling curves of the normal cock (fig. 11) showed the same divergence to a still greater extent. Here again the curves of the head appendices showed the lowest capacity for swelling. The curves of the skin samples showed the diver-

gence in a still higher degree. The distance between the extremes at pH 2.4 were more than 400%.

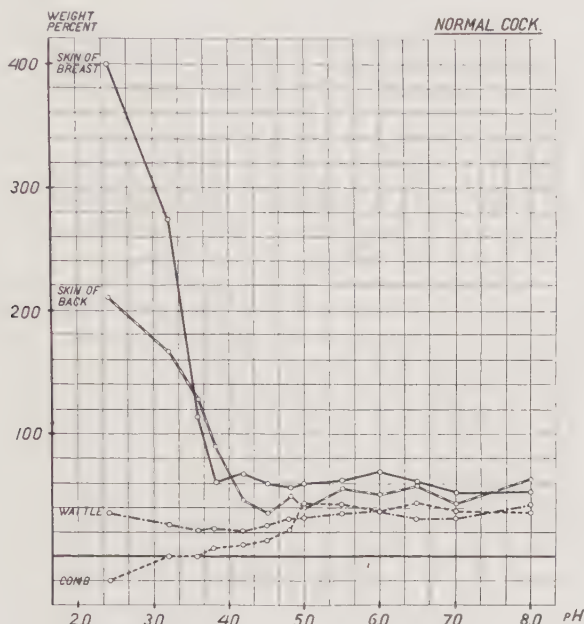


Fig. 11 Swelling curves of different skin regions of the normal cock.

DISCUSSION

1. The head appendices

The fibrillar compounds of the connective tissue in the fowl's comb are described in the literature under different names and with varying interpretations. Some authors (Romaney, '34; Elkner and Slonimski, '27, '30) describe collagenous fibers. Hardesty ('31) believed that the framework consists for the most part of reticulum, while Champy ('26) finds elastic fibrils ("tissu muco-élastique") predominantly in the cock's comb. Garrault ('34) draws attention to the fact that the fibers of the cock's comb have the staining properties of both reticulin and collagen. In the previously mentioned work of Heringa ('48), it is reported that, in the capon's comb, the fibers have a collagen-like appearance, while those of the cock's comb belong predominantly to the reticulin type.

Comparing this previous work with the results of our own investigations, we must conclude that the essential element in the connective tissue of the comb are fibers of the collagenous type. We agree with Elkner and Slonimski ('30), that elastic fibrils form only a negligible part of the structure of the comb. The collagen-like fibers show, however, some paradoxical properties. We saw that these fibers in the combs of both capons and cocks possessed nearly all qualities of collagen, e.g., double refraction and X-ray diagram, but, on the other hand, showed no capacity for swelling in acid pH (pH 2.4). There are two possibilities in interpreting these contradictory results, namely, this fiber substance is not collagen, or it is a collagen lacking the typical swelling capacity. If we accept the first alternative, we must conclude that it is a special fiber substance, differing from all known kinds of connective tissue fibers, which is unlikely. Therefore, we feel inclined to accept the second possibility and assume that there must be a factor in this tissue which suppresses the swelling capacity of the collagenous substance. There are some indications that this factor has some relation to the mucoid ground substance. First, we know that the swelling of collagen is antagonized by mucoid ground substance, and secondly, that the mucoid ground substance plays an important role in the tissue of the comb. It is very probable that the morphological and perhaps other properties of the fibers are a result of a changeable relation between the scleroproteid and mucopolysaccharide component of the intercellulum. In other words, the male hormone primarily or secondarily disturbs the equilibrium of the specific sensitive connective tissue of the comb.

The mucoid ground substance of the cock's comb has been examined by several authors (Berdnikoff and Champy, '31, '34; Garrault, '34). The most exact analyses of Garrault ('34) show that this substance may consist of chondroitin- or mucoitinsulfuric acid. Our treatment of slides with hyaluronidase is not chemically significant, as the enzyme is not specific. We cannot say anything with certainty regarding the develop-

ment of this substance. We have no proof that it is secreted by connective tissue cells, as Garrault ('34) and Hardesty ('31) believe. However, our results with the swelling of the capon's comb would allow the possibility, that this substance or its precursor is present in the non-swollen connective tissue itself, although it is not detectable histologically. As previously stated, the first morphological changes after hormone treatment occur in the area surrounding the capillaries. Hence, we conclude that the circulatory system may play an important role in this typical connective tissue reaction. There are many indications (Freund, '26; Hechter, Krohn and Harris, '42) that the circulatory system itself reacts to sex hormones. Champy and Demay ('35) have already hypothesized a connection between vasodilatation and the development of mucoid substance in the comb. Possibly this could also explain the alkaline phosphatase activity, which is present predominantly in vessel walls during the course of this process.

We do not find cellular proliferation accompanying the described changes in the comb's connective tissue in the formation or in the regression phase. There are no mitoses. Because of the histological aspect we can neither establish nor exclude the possibility that the cells play a major role in these changes, but it seems certain that they do not contribute to the process of growth. Undoubtedly a great deal of the increase in volume of the comb is due to a swelling process in a physicochemical sense. In other words, the enormous hydration of the intercellulum is centered in the mucoid ground substance, that is, it is a typical intercellular reaction. The designation, "growth by expansion," introduced by Heringa ('40) seems to fit this reaction very aptly.

2. The skin of the body

Two principal phenomena appear in the results of our swelling experiments. First, the difference between the swelling curves of the capon's and cock's skin is analogous to the

difference between young and old skin according to Heringa and Weidinger ('42). In other words, the skin of the capon reacts in the swelling as "young," in contrast with the "old" skin of the cock. Secondly, the presence of the male hormone exercises in different skin areas a seemingly paradoxical reaction. In the "steroid-sensitive area" (head appendices), there appears to be a "mucoidization" or "reticulinization" of the connective tissue, while the reaction in the rest of the skin would seem to represent a "collagenization."

We can interpret these results as meaning that the male hormone in the skin of the body leads to a ripening of the tissue or the formation of collagen. This is in agreement with some previous observations. The work of Herrick ('45) shows clearly that under the influence of testosterone propionate the collagen substance in the skin of capons increases to a great extent. This reaction might be explained by the nitrogen sparing effect of sex steroids, especially of testosterone. But we must point out that the administration of various sex steroids might have extremely variable effects on connective tissue and skin, which could also depend on the sexual specificity of these substances. The skin development in rats is similarly retarded by estrogens but promoted by androgens (Hooker and Pfeiffer, '43). However, the experiments of Loeb et al., ('39), as well as those of Wolfe et al., ('42) show that estrogens stimulate the formation of collagen in the stroma of the female sex organs. Worthy of mention are the observations of Wolfe et al., ('42) and Hamilton ('47) that the changes which occur in the connective tissue after administration of sex steroids over a long period, are identical with those occurring during the normal development and ripening of this tissue. From this one can conclude that the sex hormones are a necessary factor in the normal development of the connective tissue, and our experiments also support this conclusion.

SUMMARY

1. It was demonstrated that the fiber substance of the connective tissue of the comb has all the qualities of collagen,

except the swelling capacity at the lower pH's. It was concluded that there must be a factor suppressing the capacity for swelling.

2. During hormone therapy no changes occur in the elementary structure of the fibers of the capon's comb. Their appearance changes only morphologically, as they become separated and split into fine fibrils by the mucoid ground substance.

3. The reversible volume increase of the capon's comb after hormone treatment is due to an imbibition and loss of fluid by the ground substance in interstitial spaces. No cellular proliferation was observed during these changes.

4. Practically no alkaline phosphatase activity was found histochemically in the connective tissue of the comb. It was, however, present predominantly in the vessel walls during the imbibition and release phases. Simultaneously, vasodilatation and vasoconstriction of the comb's circulatory system were observed.

5. The swelling experiments demonstrated that the body skin of the capon has the swelling properties of young skin, while the skin of the normal cock reacts in the swelling as mature, old skin. From these results it was concluded that in this case the male hormone is a significant factor for the normal development of the skin.

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MITOTIC ACTIVITY IN THE HYPOPHYSIS OF THE RAT DURING PREGNANCY AND LACTATION

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INTRODUCTION

This paper is concerned with normal cellular proliferation and more particularly with factors which stimulate or inhibit mitotic division of cells. In those tissues where cell division occurs in the adult animal there is normally a balance maintained between cellular division and cellular destruction, but little is known of the conditions which initiate cell division for replacement of destroyed cells or stop division when an adequate number of cells is present.

The mitotic activity in the anterior lobe of the hypophysis of the rat has been utilized to learn more about these factors. It has been studied previously (Hunt, '42, '43, '46, '47a, '47b) in animals of different ages, at different periods of the estrous cycle and under such experimental conditions as ovariectomy and injection of sex hormones. For example, after injection of a sufficient amount of the female hormone, estrogen, there is an increased mitotic activity in the hypophysis, which led to the supposition that estrogen is a mitosis stimulating substance.

During pregnancy and lactation there is considerable alteration in the functional activity, not only of the reproductive system but also of other systems including the endocrine organs. The purpose of this study is to determine what effect these alterations may have on mitotic activity in the hypophysis. The activity should be increased, inasmuch as a considerable amount of estrogen is produced during pregnancy

(Beerstecher, '41). On the other hand the hypophysis of the rat does not increase in weight during pregnancy (Stein, '31); and if this may be considered indicative of a lack of mitotic activity, then it might be postulated that there is a substance which inhibits the mitosis stimulating effect of estrogen. The question thus arises whether there is a mitosis inhibiting substance and, if so, what it is.

There is also a marked change in the functional activity of the hypophysis and the ovaries during lactation. In the ovaries, except for an ovulatory cycle just after parturition, there is a cessation or greatly diminished growth of follicles and production of estrogen until after weaning. What effect does this have on mitotic activity in the hypophysis, and is the so-called mitosis stimulating effect of estrogen suppressed during this period? The observations and experiments presented here attempt to answer these questions at least in part.

MATERIAL AND METHODS

The Long-Evans strain of rats was used. Due to the limited size of the colony it was necessary to utilize animals at different seasons of the year with corresponding fluctuations in temperature. This, however, was believed not to affect the results materially. Such factors as time of day of killing, diet, and health were kept as constant as possible. Animals killed early in pregnancy were all checked for spermatozoa on the morning after mating.

Animals were killed by decapitation; and, after removing the brain and surrounding tissue, the base of the skull with the hypophysis in situ was fixed in Allen's B-15 fluid. Sections were cut at 3μ and stained in hematoxylin, acid fuchsin, and aniline blue (Masson's technique). In determining mitotic activity, all dividing cells were counted in one or more sections, the area of each being calculated with the aid of a planimeter. Thus mitotic activity is expressed as the number of dividing cells occurring in a square millimeter of section 3μ in thickness. At least 5 mm^2 of section from each gland were examined.

The estrogen used was estradiol benzoate (Progynon-B)¹ in sesame oil, and the gonadotropins used were from pregnant mare serum (Anteron)¹ and pregnancy urine (Pranturon).¹ All injections were made subcutaneously except for group 1, table 2, in which the injections were made intraperitoneally.

RESULTS

Mitotic activity in the hypophysis during pregnancy

The mitotic activity in the hypophysis at the beginning of pregnancy is essentially the same as that found during the postovulatory phase of the estrous cycle (Hunt, '42, '43). On the first day of pregnancy the average activity is 24.9 per

TABLE 1
Mitotic activity in the hypophysis during pregnancy

GROUP	NUMBER OF ANIMALS	AGE AVERAGE	PREGNANT DAYS	MITOSES PER MM ² MEAN $\pm$ S.E.
1	4	108	1	24.9 $\pm$ 10.3
2	8	103	2	10.6 $\pm$ 6.6
3	4	90	3	3.9 $\pm$ 1.8
4	15	109	4 to 21	1.1 $\pm$ 0.6
5	35	153	4 to 21	0.6 $\pm$ 0.8

square millimeter of section. This is reduced to 10.6 on the second day and to 3.9 on the third (table 1, groups 1, 2 and 3).

The animals killed on the first or second days of pregnancy invariably show a higher mitotic activity than ovariectomized animals or normal ones of comparable age killed in diestrus in which the average activity is less than 1 per square millimeter. In early pregnancy, as in the postovulatory phase during an estrous cycle, this increased activity is apparently the consequence of the increased production of estrogen by the ovary.

¹ The Progynon-B, Anteron and Pranturon were generously supplied through the courtesy of the Schering Corporation.

This conclusion is based on the observation (Hunt, '47a) that the mitotic activity in ovariectomized animals can be increased from less than 1 to an average of about 20 per square millimeter by injections of estrogen (table 2, groups 1 and 2) made on the second and third days before death.

Although the evidence is not conclusive, there is some indication that mitotic activity may persist at an increased level for a longer period in early pregnancy than it does in the post-ovulatory phase of a non-gravid cycle. During an estrous cycle increased mitotic activity may occur 30 or as much as 36 hours after proestrus, but it is not found to be increased 48 hours later when the animal is usually in the diestrus phase. On the other hand, the majority of animals killed on the second day of pregnancy or 48 hours after proestrus show an increased activity, and some killed on the third day have an activity greater than that found at diestrus.

After the third day of pregnancy an increased mitotic activity is not observed in the hypophysis until after parturition (table 1, groups 4 and 5). In the glands from 50 animals none shows more than 2.7 mitoses per square millimeter. The average for the group is 0.8 per square millimeter, which is about the same as that observed in a group killed in diestrus.

Although only a few animals were killed on each of the days of pregnancy so that a slight increase at some time might have been missed, it is believed that a sufficient number has been examined to say that a distinct increase does not occur.

The average mitotic activity is slightly higher in the younger pregnant animals of this group of 50. Those with an average age of 109 days (range 84–124 days) have an average of 1.1 mitoses per square millimeter (table 1, group 4), whereas those with an average age of 153 days (range 126–190 days) have an average of 0.6 mitoses (table 1, group 5). This age difference which was observed previously (Hunt, '43, '47a) may be accounted for by the decline in mitotic activity that accompanies advance in age.

*Mitotic activity in pregnant animals with injections
of estrogen*

A preliminary report of these experiments was made previously (Hunt, '47b), but the results are briefly included here to facilitate comparison with other observations.

Each animal of two groups of rats was given subcutaneous injections of 25 μ g of estrogen 72 and 48 hours before death. One group was 6 to 8 days pregnant when killed and the other 12 to 15 days pregnant. The animals killed earlier in pregnancy (table 2, group 3), before placentae were developed,

TABLE 2

*Mitotic activity in the hypophysis of pregnant and non-pregnant rats after
injections of estrogen and gonadotropins*

GROUP	NUMBER OF ANIMALS	AGE AVE.	OVARI- ECT. DAYS	PREGNANT DAYS	ESTROGEN INJECTED	GONADOTROPIN INJECTED	MITOSES PER MM ² $\pm$ S.E.
					μ g		
1	10	83	26		2 $\times$ 25		22.1 $\pm$ 5.5
2	10	97	37		2 $\times$ 25		20.0 $\pm$ 1.9
3	9	92	..	6 to 8	2 $\times$ 25		17.3 $\pm$ 3.3
4	13	94	..	12 to 15	2 $\times$ 25		4.4 $\pm$ 1.4
5	8	98	39		2 $\times$ 25	4 $\times$ 150 Pr.	4.9 $\pm$ 2.2
6	10	110	49		2 $\times$ 25	3 $\times$ 500 Ant.	3.8 $\pm$ 0.9

have an average mitotic activity of 17.3 which is not significantly different from the averages of the control groups (table 2, groups 1 and 2). The others, killed on the 12th to 15th day of pregnancy (table 2, group 4) have an average activity of 4.4 which is significantly lower than the average of group 3. Because the presence of well developed placentae is the main difference between these two groups, it seems probable that placental gonadotropic hormone, which is similar to the hypophyseal gonadotropin, is the factor which suppresses the mitosis stimulating effect of the injected estrogen.

Mitotic activity in ovariectomized rats with injections of gonadotropins and estrogen

Because of the possibility that gonadotropic hormone suppresses the mitosis stimulating effect of estrogen, it was injected into a group of ovariectomized animals which also received estrogen. Controls used for this series are groups 1 and 2, table 2, which have a mitotic activity of approximately 20 mitoses per square millimeter.

The 8 animals of group 5 (table 2), which were ovariectomized 39 days before death, each received 4 subcutaneous injections of 150 I.U. of gonadotropic hormone from pregnancy urine (Pranturon) in addition to two subcutaneous injections of 25 μ g of estrogen. The gonadotropin was injected 43, 52, 67, and 76 hours before death and the estrogen 48 and 72 hours before death. The average mitotic activity in the hypophysis of this group is 4.9 per square millimeter. This average is significantly different from the control groups 1 and 2 and is very nearly the same as the average activity found in the animals killed on the 12th to 15th day of pregnancy which received the same amount of estrogen (table 2, group 4).

In another group (table 2, group 6) each of 10 animals, ovariectomized 49 days before death, received, in addition to estrogen, three injections of 500 I.U. of gonadotropic hormone from pregnant mare serum (Anteron). The injections were made 24, 48, and 72 hours before death. The average mitotic activity in this group is 3.8 per square millimeter. This is essentially the same result as found in the group receiving Pranturon and is also significantly lower than the control groups which received estrogen only.

Another group was injected with smaller amounts of gonadotropin but showed either a slight suppression only or in the case of those receiving injections of 25 and 50 units no significant difference.

Mitotic activity in the hypophysis during lactation

There is a considerable increase in mitotic activity in the hypophysis in some animals on the day of parturition and also

the two following days, but there is lack of uniformity of activity in the groups. Consequently, the range rather than the average is given for some groups in table 3.

In the glands of 4 animals killed from 6 to 16 hours post-partum, the mitotic activity is 1.1, 2.0, 19.0 and 30.4 per square millimeter. In all of these animals, examination of sections of the ovaries and tubes shows that ovulation had not occurred, although certain of the follicles show growth.

Animals killed 24 to 36 hours post-partum also have considerable range in mitotic activity. Of the 7 animals with an

TABLE 3
Mitotic activity in the hypophysis of lactating rats

GROUP	NUMBER OF ANIMALS	AGE AVE.	HOURS OR DAYS POST-PARTUM	OVARIECT. DAYS	ESTROGEN INJECTED	NURSING AT DEATH	MITOSES PER MM ² RANGE OR MEAN $\pm$ S.E.
					μg		
1	4	108	6 to 16 hrs.			Yes	1.1-30.4
2	7	108	24 to 36 hrs.			Yes	3.2-43.1
3	6	208	24 to 36 hrs.			Yes	3.3-13.7
4	4	109	48 to 58 hrs.			Yes	1.0-51.7
5	4	107	72 to 85 hrs.			Yes	0.7-2.4
6	10	143	5 to 20 days			Yes	0.6 $\pm$ 0.2
7	7	112	12 to 16 days	7 to 8		Yes	0.7 $\pm$ 0.2
8	14	114	7 to 16 days	7 to 9	4 $\times$ 16.6	Yes	11.6 $\pm$ 2.4
9	13	110	12 to 16 days	7 to 9	4 $\times$ 16.6	No	4.8 $\pm$ 0.7
10	34	110		31 to 35	4 $\times$ 16.6	No	17.0 $\pm$ 1.1

average age of 108 days (table 3, group 2), three have a relatively high activity and 4 a relatively low one. In a group of 6 older animals with an average age of 208 days (table 3, group 3) two have a much lower activity than the others. Sections of the ovaries of the animals of these two groups show that ovulation had occurred.

Of the 4 animals killed 48-58 hours post-partum (table 3, group 4) one has a low and the others high activity.

Animals killed 72 hours or more post-partum invariably have a low activity in the hypophysis until ovulatory cycles are reestablished after weaning. Those killed 72 to 85 hours post-

partum have from 0.7 to 2.4 mitoses per square millimeter; and the 10 animals killed on the 5th, 7th, 8th, 10th, 14th, 16th, and 20th days of lactation have from 0 to 1.9 mitoses per square millimeter (table 3, groups 5 and 6). Another group of 7 animals (table 3, group 7) which were killed on the 12th, 13th, 15th, and 16th days of lactation and which had been ovariectomized 7 or 8 days before death likewise have a low level of mitotic activity.

Inasmuch as an ovulatory cycle and increased mitotic activity in the hypophysis apparently do not occur during lactation, except just after parturition, the question arose whether there is a mitosis suppressing factor present during lactation comparable to that of pregnancy. Accordingly, 14 lactating animals (table 3, group 8) which had been ovariectomized 7-9 days previously, were injected with estrogen. Each animal received an injection of 16.6 μ g 28, 36, 52, and 60 hours before death. The animals were killed on the 7th, 12th, 13th, 15th, and 16th days of lactation and at that time were nursing three to 8 young (average number 5.7). The average mitotic activity in this group is 11.6 per square millimeter. This average is significantly less ($P < .01$) than the average mitotic activity of 17 per square millimeter found in a group (10, table 3) of ovariectomized animals of about the same average age which received the same amount of estrogen.

In another group, 13 animals (table 3, group 9), which were killed on the 12th, 13th, 14th, 15th, and 16th days of lactation, received the same amount of estrogen; but the young were removed 60 hours before death of the mothers. In this group the average mitotic activity is 4.8 per square millimeter. This is significantly less than the average of group 8, although the only important difference between the two groups was the presence in group 8 of nursing young.

DISCUSSION

The low mitotic activity in the hypophysis throughout all but the first few days of pregnancy is difficult to understand if estrogen is a mitosis stimulating substance, because during

the first two weeks of pregnancy there is a very considerable increase in estrogen judging by that found in the urine (Beerstecher, '41). Even when this normal production is augmented by injections during mid-pregnancy, there is no rise in activity comparable to that found in ovariectomized animals of the same age which received the same amount of injected estrogen. Thus one might postulate that there is a substance produced during pregnancy which either inhibits the action of estrogen or acts by suppressing mitosis.

The presence of corpora lutea and the placentae during pregnancy suggests that the mitosis suppressing substance may be either progesterone produced by the corpora or gonadotropin produced by the placentae. In favor of progesterone is the report by Schilling and Laqueur ('41) that the increase in pituitary weight following injections of estrogen over a 10-week period can be suppressed by the simultaneous injection of progesterone. Against it, however, is the fact that there is no marked suppression in the mitosis stimulating effect of estrogen during pregnancy if it is injected before the placentae are established, even though the corpora lutea at this time are active in producing progesterone. Furthermore, no significant suppression in mitotic activity was noted after injecting progesterone and estrogen simultaneously (Hunt, '46). In this experiment 50 μ g of progesterone (Proluton) were injected daily on the 5 days preceding death, and 250 μ g of estradiol benzoate were injected 48 and 72 hours before death. The average mitotic activity in the hypophysis of this group was 30.7 per square millimeter compared with an average of 30.9 per square millimeter for the group receiving only the same amount of estrogen. Thus at these dosage levels, progesterone does not counteract the effect of estrogen.

There is more evidence to support the idea that the placental gonadotropin, which is similar to that produced by the hypophysis, is the suppressing substance. Not only does a marked suppression of the mitosis stimulating effect of estrogen occur only after the placentae are established, but suppression can be effected by injecting gonadotropins along

with estrogen into ovariectomized animals. It is true that large amounts of gonadotropins are required for the suppression, but this may be due to the fact that they were obtained from a different species. Furthermore, recent experiments indicate that a greater suppression may be obtained with a smaller amount of material if injections are made nearer the time of death.

At parturition, with the loss of the placentae and removal of the source of the placental gonadotropins, conditions should be such that the hypophysis could respond once more to the mitosis stimulating effect of estrogen. It appears to do so because during the first few days after parturition there is a greatly increased mitotic activity in the hypophysis. Usually, during an estrous cycle, mitotic activity attains the highest level in the hypophysis some 12 to 24 hours after ovulation. In post-parturient animals, however, a high level of activity may be attained before ovulation occurs. This raises the question of whether estrogen is actually a mitosis stimulating substance. It seems more likely that it acts by regulating in some way the level of gonadotropin present in the hypophysis. It may regulate the level by inactivation, according to Jungeck, Heller and Nelson ('47) or by causing a release of the secretion from the cells and subsequent excretion (Severinghaus, '39); but, however it acts, the fact seems to be established that gonadotropin is greatly diminished after there has been a high level of estrogen circulating in the body. At the time when there is a low level of gonadotropin many of the cells of the hypophysis, especially the chromophobes, divide mitotically. During pregnancy the placental gonadotropin presumably augments that produced in the hypophysis, and consequently mitotic activity does not occur even though considerable estrogen may be present. Thus the main factor in determining mitotic activity in the hypophysis is the amount of gonadotropin or other secretion present in its cells, or it might be better to say that the determining factor is the amount

of these secretions present in the body, because the placental gonadotropin or injections of gonadotropin are likewise effective in regulating mitotic activity.

The lactogenic secretion of the hypophysis, as well as gonadotropin, is also a factor which regulates mitotic activity. This would explain the low level of activity during lactation subsequent to the initial increase after parturition. In the rat hypophysis the lactogen content attains a high level by the third day post-partum (Hurst and Turner, '42). This rise to a high level is believed by Meites and Turner ('48) to be due to the large amount of estrogen at this time. Later in lactation estrogen apparently has nothing to do with lactogen secretion in the hypophysis, but suckling has a marked effect (Selye, '34, Reece and Turner, '37). The latter workers showed that if the young were removed 15 hours before killing the nursing mother, the lactogen content of the hypophysis was significantly higher than in the mother allowed to suckle its young. This may explain the observation that after injections of estrogen, mitotic activity was markedly suppressed during lactation if the young were removed at the time the first injection of estrogen was made, i.e., 60 hours before killing the mother. The suppression is believed to be due to the greater lactogen content which is the result of the lack of the suckling stimulus.

SUMMARY AND CONCLUSION

1. After the first three days of pregnancy, mitotic activity in the hypophysis of the rat persists at a low level until after parturition.
2. The apparent mitosis stimulating effect of estrogen produced during pregnancy or supplied by injection is suppressed after the placentae are developed but not before.
3. Injections of gonadotropins suppress the mitosis stimulating effect of estrogen in ovariectomized females.
4. During lactation there is an increased mitotic activity in the hypophysis on the first three days post-partum, but on the 4th day it falls to a low level which is maintained until after weaning.

5. There is a moderate suppression of the mitosis stimulating effect of injected estrogen during lactation and an even greater suppression if the young are removed from their mother 60 hours before the mother is killed.

6. It is concluded that the lactogenic hormone of the hypophysis, which has been shown by others to be increased by preventing the young from suckling, is the factor which suppresses the mitosis stimulating effect of estrogen during lactation.

7. It is also concluded that estrogen is not a direct mitosis stimulating substance but acts indirectly by regulating the level of gonadotropin in the hypophysis. Further, the important factor which determines mitotic activity in the hypophysis is the level of secretion produced by it or, in the event of pregnancy, the similar secretion produced by the placentae.

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HISTOCHEMICAL MEETING

On March 25, 1950 a meeting of anatomists, bacteriologists, biochemists, pathologists, and others interested in histochemistry as a method of study in their respective disciplines will convene in the Department of Anatomy of the University of Pennsylvania, Philadelphia, Pa. A considerable number of papers have already been promised. Further information and programs may be obtained by writing Dr. R. D. Lillie, National Institutes of Health, Bethesda 14, Maryland.

MORPHOGENETIC STUDIES OF THE RABBIT

VII. AORTIC ARCH VARIATIONS IN RELATION TO REGIONALLY SPECIFIC GROWTH DIFFERENCES ¹

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TWO FIGURES

INTRODUCTION

In previous communications (Sawin, '46 and Sawin and Nace, '48), evidence was found that skeletal variations at the thoraco-lumbar border and both skeletal and vascular variation at the lumbo-sacral borders of the axial skeleton serve in a general way to define the limits of growth centers of different magnitudes in each of several closely bred races of rabbits. In further attempts to delimit the area of influence of these centers, it has been of particular interest to consider the variation in pattern of the aortic arch. Such variation is both intra-specific and inter-specific. The varieties found in man have been discussed voluminously, dating back to the 15th century (Poynter, '16). The types commonly found in mammals were described by Cuvier (1810), and the most comprehensive study is that of Parsons ('02), who first suggested that such variations might depend upon body build rather than systematic relationships. Both phylogenetically and ontogenetically, the mammalian arch is recognized as a persistent 4th member on the left side in a series of 6 symmetrical pairs of arches. In

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birds it is the right arch which persists and the lower vertebrates (particularly in the lower categories) characteristically retain a more complete set. Variations resembling that of the bird, as well as combinations of both right and left arches in one individual have been reported occasionally in mammals. One of the first anomalies to be described in the rabbit was one of the former type (Legallois, 1824). Of more frequent occurrence are anomalies involving the carotid and subclavian arteries which correspond to remnants of two of the adjacent arches of the early, and in general, transient embryonic pattern. Such anomalies can be readily explained as irregular persistence or atrophy of parts of the embryonic pattern. Fusion of adjacent parts may also influence the pattern (Arey, '30), see also Baldwin ('20). Such an interpretation does not contribute to knowledge of the fundamental physiological processes on which the changes are dependent, although the general consensus seems to be that the adult arrangement of vessels is a result of mechanical factors modifying the usual habit of development, an opinion which goes back to Baader (1866), cited by C. W. M. Poynter ('16). Parsons ('02), in a comparative study of the arches, concluded that the type of arch was in some measure correlated with the breadth of the thorax and its proximity to the superior thoracic aperture. Lockhart ('30) suggests that a shift of influence determines the selection of parts of an anastomosing arterial network, some vessels of which enlarge while others degenerate through disuse. Such a process, operating by chance alone, affords unlimited possible variations. As Blincoe et al. ('36) remark: "the marvel is that the so-called normal arrangement is produced with such uncanny similarity so regularly and that such delicately adjusted processes so seldom go wrong." This stability suggests that the processes involved are hereditary, and that abnormal types either are the direct result of unusual environmental circumstances, or are in some way due indirectly to minor fluctuations in one of two or more genetic processes whose interaction does not quite achieve the proper threshold for complete fulfillment. Disastrous effects of re-

tardation at critical stages or of inadequate supplies of vitamin A, etc., upon the vascular system have been described by Wilson ('48). Since maximum growth can only be achieved by an optimum supply of both food and oxygen, the interrelation between vascular development and growth is obvious. The present study, therefore, is not only a study of the inheritance of variations in the pattern of the vessels arising from the aorta, but because of previous knowledge of differences in regional growth in the inbred races of rabbits used, it affords an opportunity to determine whether or not such regional growth differences are associated with any given pattern to a greater or lesser degree than with another.

MATERIALS AND METHODS

The specimens used in this study were for the most part the same animals used in earlier studies (Sawin, '45, '46 and Sawin and Nace, '48) and prepared either by gross dissection of adults or newborn or by injection through the ventricles with colored vinylite with subsequent clearing with KOH and glycerine. The same three races, III, V, and X, were used and, as previously, animals were raised under as nearly a uniform environment as possible, and matings were based upon the presence or absence of an extra or 13th pair of ribs. In the earlier portion of this study, all specimens were read by H.W.E., but later the specimens were read by P.B.S. Thus, a slight error in results may have arisen as a result of this personal factor, but it is believed that it is very small.

OBSERVATIONS

Twenty types of variation have been observed in the aortic arch of the rabbit. Fifteen of these were described by Edmonds and Sawin ('36) and are shown in figure 1.

The most common type (normal) in rabbits is the A type in which the brachiocephalic and innominate arteries are of approximately equal length and prominence. Type C lacks a brachiocephalic artery. Types B', B'', and E all possess a nor-

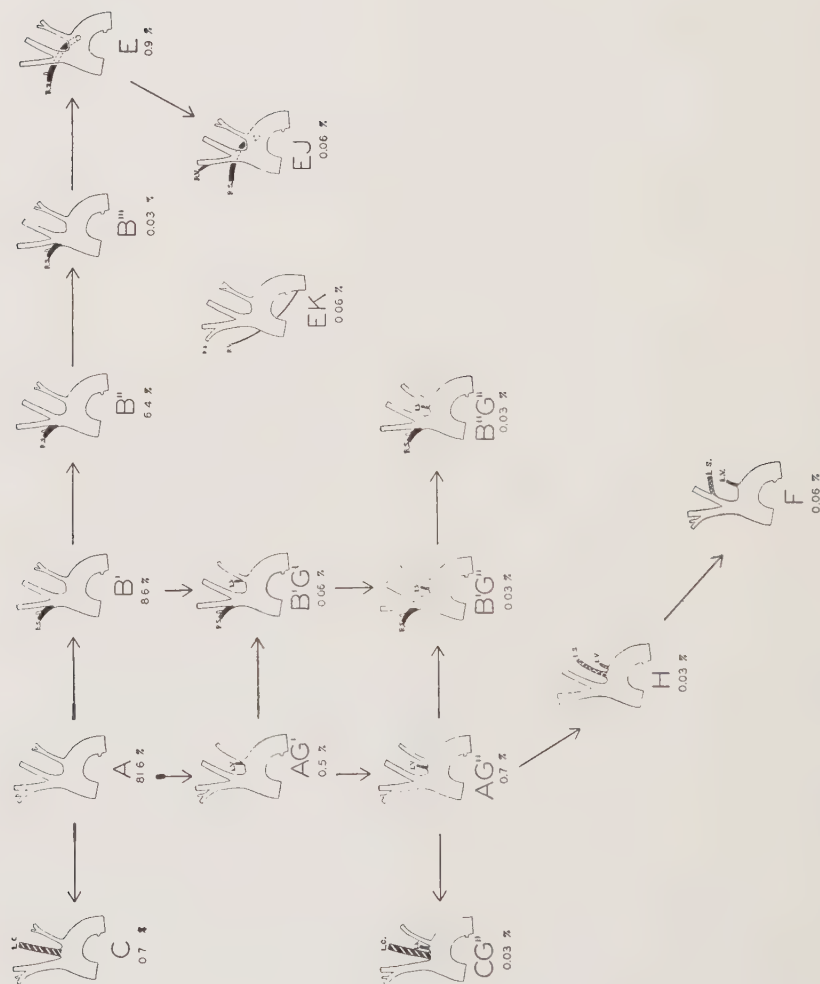


Fig. 1 Incidence of types of aortic arch found in postmortem examination of 3000 rabbits.

mal brachiocephalic artery, but due to the posterior migration of the origin of the right subclavian artery to or below the level of the bifurcation of the brachiocephalic artery, the innominate artery is reduced or absent. Such reduction in the rabbit was reported as early as 1868 by Krause. In type E, which is well known in man and commonly designated as a retroesophageal right subclavian artery (R.R.S.A.) the right subclavian takes its origin from the aortic arch as its last branch, and passes dorsal to trachea and esophagus to reach its point of termination. It is commonly supposed that the R.R.S.A. represents a persistent portion of the right dorsal aorta of the embryo. Specimens of this type were reported in the rabbit by Kussmaul and Tanner (1857), Smith (1891), White (1893), and Eales ('27). Types EK and EJ are variations of the E type in which the origin of the right vertebral artery is also varied and the G' and G'' and H variations in which the origin of the left vertebral artery is varied.

The incidence of each of these types among the first 3000 rabbits as typed by H. W. E., are shown in figure 1. For the purposes of this study the subtypes involving the vertebral arteries were not differentiated. Since 1936 it was found convenient to record 4 additional types, Asb (intermediate between C and A), A- (intermediate between A and B'), and combination types A-sb and B'sb.

Mating of specific types

As was the case in early studies of rib variation, the preliminary study of arch variations reported by Edmonds and Sawin ('36), seemed to indicate some approximation to typical mendelian proportions between normals and certain of the abnormal types, but like the rib variation, continued study has demonstrated a more complex background. In table 1 are shown the data obtained from matings of known specific type. It is seen that although matings of the C type (lacking a brachiocephalic artery) (mating 1, table 1) produce their own type in a majority of instances, they also produce Asb, A, and

even A-. Although Asb type reproduces itself in 29% of cases, it produces a few C's (8.3%), and 62% of A type. A types (mating 6) produce the entire range of variation, of which 69% are like themselves. The progeny obtained from B types is too small to be of great significance, but it is important to note that they also produce the normal type. Mixed matings (especially matings 2, 4, 5 and 9) in general appear to produce a greater range than those of like types, but a notable tendency

TABLE 1

Per cent incidence of aortic arch types in progeny of parents of specific arch types. Type A is regarded as the normal type. Types deficient with respect to the brachiocephalic artery are shown to the left. Those deficient in the innominate are shown to the right, and type E involving the R.R.S.A. to the extreme right.

MATING	DEFICIENT BRACHIOCEPHALIC			NORMAL	DEFICIENT INNOMINATE			R.R.S.A.	TOTAL
	No. of matings	C	Asb		A	A-	B'		
1. C × C	3	58.3	16.6	16.6	8.3				12
2. C × Asb	21	22.6	14.3	51.8	4.8	6.0	.6		168
3. Asb × Asb	7	8.3	29.1	62.5					48
4. C × A	14	7.9	27.6	53.9	4.0	5.3	1.3		76
5. Asb × A	33	3.4	14.3	69.5	.5	9.4	3.0		203
6. A × A	42	4.9	10.9	69.4	.4	8.5	3.9	2.1	284
7. Asb × A-	4		3.4	82.7		13.6			29
8. A. × A-	4		9.0	66.6		24.2			33
9. A × B	14		3.0	66.6	.3	15.0	14.3	.6	300
10. B × B	1			40.0		20.0	40.0		5
11. B × E	1			33.3		66.6			3

is found for the distribution to be centered to the left in the tabulation in matings of deficient brachiocephalic arteries; to the right in matings of deficient innominates; and to cover a greater range in matings of the two extremes or of normals with either extreme. Dominance of one type over another, and segregation according to the usual mendelian ratios are not apparent. Furthermore, the continuity of types, although neither perfect nor strictly symmetrical in the tabulation, is more like that which is considered typical of quantitative inheritance in which several factors are involved. Thus, it ap-

pears as if the two major abnormal types (reduced or absent brachiocephalic or reduced or absent innominate) may be manifestations of similar fundamental influence as found in ribs, ventral spinous processes, etc.

Patterns in inbred races

The first evidence of the relation of the aortic arch variations to the regional growth differences previously described is found in the inbred races themselves.

In table 2 and group A, figure 2, are shown the distributions of the aortic arch types in populations of races III (mating 1), V (mating 3) and X (mating 2), each of which population is known from previous studies to possess a specific vertebral

TABLE 2
Per cent distribution of aortic arch patterns in 5 races of rabbits

MATING	E	A	A-	B'	B''	B'''	A-sb	B'sb	Asb	C	TOTAL
III	..	17.3	4.0	8.0	..	..	1.3	7.3	33.3	28.7	150
X	..	58.0	6.7	10.8	3.0	..	..	.4	16.4	4.8	269
V	.1	65.0	7.0	18.1	3.8	..	.2	.1	5.5	.1	853
V466	1.1	26.5	12.1	39.7	12.6	3.4	.6	..	4.0	..	173
IIc	4.1	74.1	..	6.1	6.8	.7	..	..	6.8	1.4	147

pattern. In this table and figure, as well as those which will follow, the data are arranged in a slightly different order, from left to right, normal (both innominate and brachiocephalic present), innominate deficient, and finally brachiocephalic deficient, which as will be shown later, is according to the antero-posterior level at which the variations occur. When thus arranged, each population tends to fall in a continuous but asymmetrical distribution which is different in each case. Although this continuity must be kept in mind, it will be convenient in describing races to refer merely to the proportion of normals, of innominate deficient or of brachiocephalic deficient as shown by the individual figures. Race III (mating 1) characteristically has a short or no brachiocephalic artery

(60% of cases). Twelve per cent of its individuals manifest a short innominate, and only 17% are of the recognized normal type.

In contrast, race V (mating 3, table 2 or figure 2 A) has 65% of the individuals with the normal A type. Six per cent are deficient in the brachiocephalic artery but 28% are deficient in some measure in the innominate.

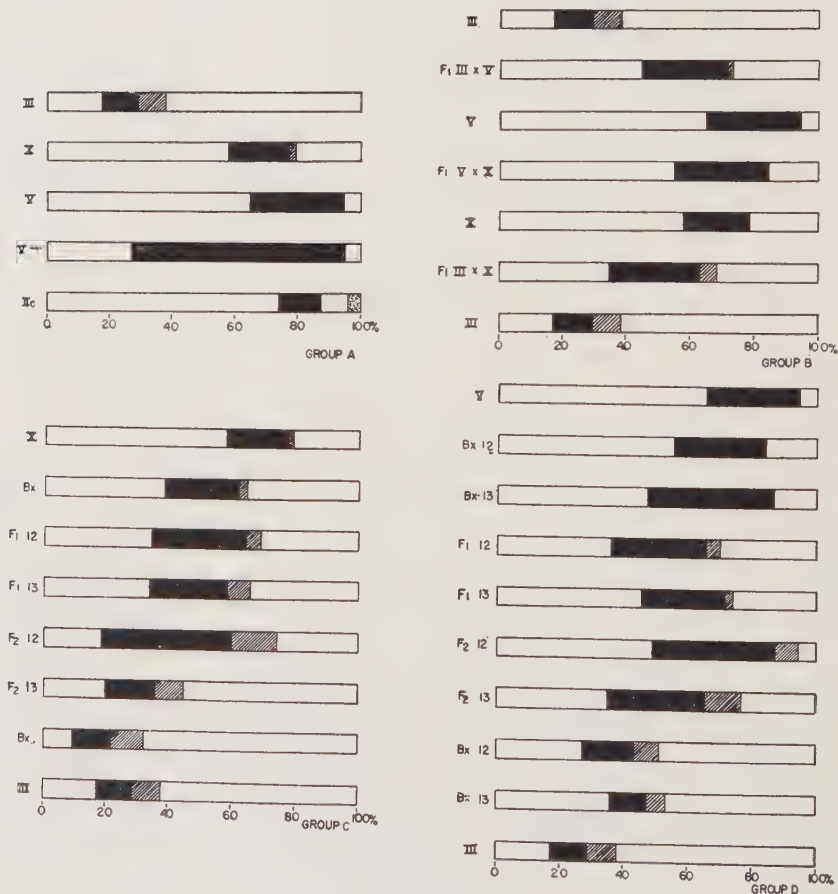


Fig. 2 Per cent distribution of the 4 major types of aortic arch pattern. Normal type (A) at left; deficient innominate (A-, B' and B'') in black; deficient brachiocephalic (Asb and C) to right; combination types (cross-hatched), and type E, the retroesophageal right subclavian artery (stippled).

Race X (mating 2, table 2 or fig. 2 A) likewise has a high per cent (58) of normal A type individuals and the abnormal types are about equally divided, 22% deficient in brachiocephalic and 20% in the innominate.

Thus, race III characteristically manifests the more posterior deficiency, race V the more anterior deficiency, and race X neither one more than the other.

One subline of race V is of interest because continued selection for 5 generations resulted in a population with a much higher proportion of deficient innominates. Progeny were selected to be parents for each generation from parents which had previously produced higher proportions of the B type of arch, and the data shown were taken from the last two generations. They show approximately 70% of innominate deficient individuals as compared with the general population of race V (see mating 4, table 2). One more race (IIc) is shown in table 2 (mating 5). This race had the same common origin as race V, but when examined for the arch pattern, after approximately 5 generations, its members characteristically produced a very high proportion of A type arches as compared with the other races, and also the largest proportion of the E type arch (which represents a persistent portion of the right dorsal aorta of the embryo). Sufficient data on patterns of the aortic arches in crosses of these two races unfortunately are not available. These distinct racial differences clearly indicate the genetic background of the variation.

Pattern of distribution of F_1 hybrid generations

The distribution of patterns in the hybrid generations derived from crossing these races is shown in table 3 (matings 2, 4 and 6), and in figure 2, group B. In each case the position of the central tendency of the distribution of the F_1 tends to be shifted to the right or left in such a manner as to fall approximately intermediate between its comparable positions in the parental races. In the III $\times$ V cross (mating 2), the proportions of short brachiocephalic, normal and short innominates

are approximately intermediate between those of each type of the parental races. The same is true in the $V \times X$ F_1 's. Race X is quite different from race III, and while in the F_1 's of the $III \times X$ cross the proportions of deficient brachiocephalics and of normals likewise tend to be intermediate, the distribution is such that the number of deficient innominate arteries of the several kinds exceeds either of the parent races by more than 10 or 20%. This tendency for the F_1 in most cases to be intermediate but in some cases to exceed either parent is the same sort of phenomenon noted by Sawin ('46) and Sawin and

TABLE 3

Per cent distribution of aortic arch patterns in races III, V and X and in their F_1 hybrid progeny

MATING	R.R.S.A. NORMAL		INNOMINATE			COMBINATION		BRACHIO- CEPHALIC		TOTAL
	E	A	A-	B'	B''	A-sb	B'sb	Asb	C	
1. III	..	17.3	4.0	8.0	..	1.3	7.3	33.3	28.7	150
2. F_1	..	42.5	8.9	17.1	2.1	2.1	0.7	21.2	5.5	146
3. V	.1	65.1	7.0	18.1	3.8	0.2	0.1	5.5	0.1	853
4. F_1	..	56.5	5.8	20.3	1.4	..	..	10.1	5.8	69
5. X	..	58.0	6.7	10.8	3.0	..	0.4	16.4	4.8	269
6. F_1	..	34.3	11.6	14.9	2.2	1.9	3.4	22.8	9.0	268
7. III	...	17.3	4.0	8.0	..	1.3	7.3	33.3	28.7	150

Nace ('48) in connection with studies of ventral spinous processes and iliolumbar arteries, and it is of further interest to determine what happens to this accumulated deficiency in the innominate artery in subsequent generations. Before turning to these, however, it should be noted that when the F_1 's of the $III \times X$ cross are separated into two populations (mating 3 and 4, table 4) on the basis of possession of 12 or 13 ribs, it is seen that 12-ribbed F_1 's tend to have the greater number lacking in the innominate arteries, whereas 13-ribbed have the greater number lacking in the brachiocephalic. The same is true of the F_1 of the $III \times V$ cross (matings 4 and 5, table 5), except that in this cross it is the normal type which is largest in 13-ribbed F_1 's rather than the brachiocephalic deficient

types. In general, there appears to be a tendency for more innominate deficient types to be produced throughout. This fact is of considerable significance, as will be seen later.

Pattern distribution of F_2 and backcross generations. In table 4 (and in fig. 2, group C) are shown the distributions of the F_2 (matings 5 and 6) and backcross populations (matings 2 and 7) of the cross of races X and III in relation to the parent populations (matings 1 and 8) and the F_1 (matings 3 and 4). Here in the total F_2 are found a much higher proportion of deficient brachiocephalics, particularly of the Asb type, than in the F_1 or in the backcross to race X, and a corresponding decrease in normals and the deficient innominate types A-, B'

TABLE 4

Per cent distribution of aortic arch patterns in the cross of races III and X

MATINGS	NORMAL	INNOMINATE			COMBINATION		BRACHIO- CEPHALIC	TOTAL
	A	A-	B'	B''	A-sb	B'sb	Asb C	
1. X	58.0	6.7	10.8	3.0	..	0.4	16.4	269
2. Bx ^x (12)	38.4	10.6	12.6	1.3	0.7	1.3	26.5	151
3. F ₁ (12)	34.5	13.6	15.3	1.7	1.1	3.4	23.2	177
4. F ₁ (13)	34.1	7.7	14.3	3.3	3.3	3.3	22.0	91
5. F ₂ (12)	18.6	12.8	9.3	..	10.5	3.5	33.7	86
6. F ₂	20.0	10.8	5.0	0.8	4.2	4.2	45.8	120
7. Bx ^{III}	9.5	6.0	6.5	0.7	3.0	7.7	50.0	168
8. III	17.3	4.0	8.0	..	1.3	7.3	33.3	150

and B''. It is again interesting to note that, particularly as compared with the F_1 , 13-ribbed individuals (mating 6) have the greater proportion of deficient brachiocephalic arteries and less of the deficient innominate types. It is also important to note the greater incidence of the combination types A-sb and B'sb in the two F_2 populations.

Both backcrosses (matings 2 and 7) show a tendency for their distributions to return to that of the particular parent race concerned. The Bx^{III} particularly resembles race III in possessing the exceedingly high proportion (66%) of brachiocephalic deficient individuals, and there is also an increase in the number of combination types as in the F_2 .

Similarly in table 5 (and in fig. 2, group D) are shown the distributions for races III (mating 10) and V (mating 1) and their hybrid generations. The same intermediate types of distribution are to be noted for each generation. Again, as in the F_2 ($III \times X$) there is to be noted a general increase in type A or normal and particularly the innominate deficient types A- and B', and a decrease in the deficient brachiocephalic types. Of special significance is the fact that this tendency is more pronounced in the 12-ribbed progeny, and that in the 13-ribbed progeny there are larger numbers of brachiocephalic deficient types and fewer normal and innominate deficient

TABLE 5

Per cent distribution of aortic arch patterns in the cross of races III and V

MATING	R.R.S.A. E	NOR- MAL A	INNOMINATE			COMBINATION		BRACHIO- CEPHALIC		TOTAL
			A-	B'	B''	Asb	B'sb	Asb	C	
1.V	0.1	65.1	7.0	18.1	3.8	0.2	0.1	5.5	0.1	853
2. Bx(12)	0.5	57.8	9.9	14.3	1.8	..	0.6	13.7	1.9	161
3. Bx(13)	..	47.8	7.6	24.5	5.4	0.5	0.5	9.8	3.8	184
4. F_1 (12)	..	36.0	2.0	28.0	2.0	2.0	2.0	24.0	4.0	50
5. F_1 (13)	..	45.5	13.1	11.1	2.0	1.0	1.0	20.2	6.1	99
6. F_2 (12)	..	49.0	21.6	13.7	2.0	7.8	..	5.9	..	51
7. F_2 (13)	..	34.3	16.0	13.1	1.9	6.5	4.7	22.1	1.4	213
8. Bx(12)	..	27.0	8.1	8.1	..	..	8.1	32.4	16.2	37
9. Bx(13)	..	36.0	3.6	8.3	0.6	1.2	4.1	33.7	12.4	169
10. III	..	173	4.0	8.0	..	1.3	7.3	33.3	28.7	150

types. This is in contrast to the F_1 ancestral populations (matings 4 and 5, table 5) in which there is the higher proportion of normal type among the 13-ribbed progeny.

Of particular significance is the fact that the F_2 and back-cross populations of this cross as well as the F_1 all show a greater incidence of the more normal and innominate deficient types than in the comparable matings of the $III \times X$ cross.

In the backcross to race V of the $III \times V$ cross, (matings 2 and 3, table 5), the normal types are relatively in excess in the 12-ribbed group, but in the 13-ribbed population, the innominate deficient types are in larger proportions. In the Bx^{III}

the relationships between 12 and 13 ribbed individuals compares with that of the F_1 . In both backcross populations there is the same tendency to be intermediate between that of the F_1 and the particular parent race concerned.

In summary, it may be said that the difference in distribution of types between races V and X are reflected in all of the hybrid populations with the major differences being associated with differences in the number of ribs, 12-ribbed populations tending in general to show a higher proportion of normal or innominate deficient types and 13-ribbed tending to manifest higher proportions of brachiocephalic deficient types. The exceptions to this being the F_1 of the III $\times$ V cross.

DISCUSSION

The concept of growth as a determining factor in morphological patterns is not new, and particularly is this true in connection with studies of vascular patterns. Parsons ('02) in a comprehensive systematic study, regarded the type here described as Asb as the most common mammalian type, and recognized the other variations as ranging in two directions, either towards concentration, as in our type A (forming a true brachiocephalic trunk), or towards separation, as in our type C (three of 4 separate branches of the arch). Parsons considered the immediate cause of concentration to be compression from side to side and the cause of separation to be increased breadth of the thorax. Congdon ('22) has discussed the influence which growth, relative position and blood current have in transforming the arches of man. Hammond ('37) is convinced that variations in these factors at some stage or stages in development cause a corresponding divergence in the ultimate expression of form. He believes that the aortic arches reach their final form and position as a result of growth of the organism as a whole, and that intrinsic factors play no part in this process. As he describes the arch formation in the calf, for example, "The position of the *left* subclavian changes during the 17-22 mm stages from a point well below the pulmonary arch; upward along the dorsal aorta over the arch and

thence to its final location on the arteria brachiocephalica. The short bicarotid stem does not put in an appearance until about 25 mm. With the sinking of the heart and the incorporation of the aortic trunk into the anterior heart wall as the arch level sinks, there is consequently a drawing together of the upper portions of the arches. This results in a fusion of the carotid stems, or a 'concentration of aortic arch branches' according to Parsons, thus producing a common stem of origin," (the brachiocephalic). This being true it appears that brachiocephalic deficient and innominate deficient types described in this paper are probably due to a difference in the amount of "posterior migration" of the heart relative to adjacent structures. This raises a question with respect to this very interesting phylogenetic and ontogenetic phenomenon. It is a well known fact that historically the position of the heart is relatively anterior in the lower vertebrates, as in embryos of higher vertebrates. Progressively the heart takes a more posterior position, until in mammals it finds its final location in the thoracic cavity. Whether this is an actual migration of the heart, or whether it is a relative change due to elongation of the vertebrae and associated structures pushing the head farther from the original point of attachment of the heart, is a question which needs further consideration (see Arey, '40).

If the variations in aortic arch pattern described in these races of rabbits are considered from this point of view, certain very interesting relationships are noted. Race X, with a high proportion of normal (A type) arches, and small, equal proportions of the two deficient types of arch, is the race which Baumgartner and Sawin ('43) found (in comparison to race III) to have an early accelerated and anterior onset of ossification of the ribs. Since race X seldom has extra ribs (Sawin and Hull, '46) and its ventral spinous processes are relatively poorly developed and anterior (Sawin, '46) it was concluded that race X has an enlarged or accelerated growth area localized somewhere in the anterior thoracic region. This unusual growth would explain the high proportion of normal aortic

arches with well developed innominate and brachiocephalic arteries.

Since race X also has a considerable portion of individuals with deficient brachiocephalic arteries, it would have to be assumed (following this line of reasoning) that these are individuals in which the accelerated area did not carry quite far enough posterior to insure the complete development of this artery.

Race III, which is so definitely lacking in a brachiocephalic artery in such a high proportion of cases (as compared with races V and X) is the race which Baumgartner and Sawin found to have a delayed onset of ossification of the ribs, particularly anteriorly, and presumably this retarded growth is general in the anterior thoracic region of this race. Once ossification (and presumably growth generally) does begin more posteriorly, there is good evidence (Sawin et al., see Sawin and Nace, '48) to show that as compared with either races V or X, there is a much more extensive region of general enlargement including both the thoraco-lumbar and lumbo-sacral borders. It seems probable that if Hammond's interpretation of the patterns found in the calf also applies to the rabbit, the relative position of the heart, and the right subclavian artery in race III is anterior to this accelerated growth area in a region which, as compared with the other races, is more or less retarded.

Little is known about the early rib ossification of race V but because of its high proportion of 13-ribbed individuals and magnitude and location of its ventral spinous processes it was previously concluded that an enlarged thoraco-lumbar growth area extended some distance anterior to the thoracolumbar border. Upon finding now that this race also has a large proportion of A type arches and is particularly characterized by a relatively high proportion of innominate deficient types it seems probable that if these vascular variations are associated with the previously recognized growth area of this race that area must cover in part the same region as that in race X. In other words, elongation of the axial skeleton and

associated structures at the time the limb bud was being established has proceeded so rapidly that the limb and the subclavian artery is attached more posteriorly resulting in innominate deficiency.

Since the C and Asb appear most numerous in both races III and X, which are known to be retarded in the posterior thoracic region, and because evidence was previously found to show that the accelerated growth area of V, in which the C and Asb types are almost nil, extends farther anteriorly than in III it seems reasonable to suggest that the high proportion of innominate deficient types (A-, B' and B'') in race V is due to a relatively accelerated growth of the axial skeleton, in relation to the heart and the developing limb buds in the mid-thoracic region, at the time when the limb bud and its circulation are being established. Further evidence that this is a sound interpretation is found in the hybrid generations.

Attention has already been called to the fact that in the F_1 's of all crosses the distribution of the populations into the 10 classes described tends to be intermediate between those of the two parent races, with the exception that in one cross ($III \times X$) there is a greater proportion of deficient innominates than in either parent race. This happens to be the cross in which previous studies indicate that the enlarged growth areas of the two races are so located that they probably overlap very little if at all. When combined in an F_1 cross the extreme anterior and extreme posterior types might be expected to be large because of the parental contributions, but combination might well result also in sufficient overlapping of growth stimuli that a large proportion of the innominate deficient types would result. In this case it is well to point out that this is more pronounced in the F_2 12-ribbed progeny than in the 13-ribbed which tend to resemble more closely race III. In the F_1 $V \times X$ in which there is overlapping of the growth areas, the proportion of innominate deficient types is intermediate between the proportions found in the two parent races. Further, as will be shown later, the incidence of brachiocephalic deficient types as compared with the $III \times X$ cross, which is

the lowest found in any F_1 population, is further evidence that the anterior limit of the accelerated area of race V is more anterior than that in race III.

Additional evidence in this connection is the fact that whereas the F_2 of both the $III \times V$ and $III \times X$ crosses show a pronounced increase of the combination types Asb and B'sb, they are most numerous among 13-ribbed progeny in the $III \times V$ F_2 and among the 12-ribbed progeny of the $III \times X$ F_2 (see tables or histograms group C and D, fig. 2). Similar threshold differences of this kind are found in several of the backcross generations, for example in the $III \times X$ cross, where in mating 7 (table 4) the Bx^{III} has a greater proportion of deficient brachiocephalic arteries than either parent race or F_1 . Similar cases of this type were found in the studies of both the vertebral processes (Sawin, '46) and the ilio-lumbar arteries (Sawin and Nace, '48). In the case of the vertebral processes, particular attention was called to the fact that the relative anterior-posterior position of these processes seemed to have much to do with the relative degree of their development. Whether it is strictly a matter of the antero-posterior level or whether it is more specifically a matter of the proximity of the variation to one or more areas of accelerated growth or even proximity to a point where the influences of two such areas overlap, is a question which cannot be answered at this time.

Having found evidence of relationship between these variations and the regional growth areas, it is of further interest to consider whether the variations also may help to define the limits of the growth areas. It would seem that the nature of their frequency distribution should indicate in some degree the limits of the growth areas. As was found to be the case in the study of the ilio-lumbar arteries, the pattern of blood vessels (aortic arch patterns) is not as satisfactory as skeletal units for determining the limits of these growth areas because of the non-metameric nature of vascular variation. Nevertheless the present observations combined with information from previous studies do help to restrict the limits of these recog-

nized growth areas. For example, the C and Asb types are found most numerous in races III and X in which races there is considerable reason to believe that relatively retarded growth areas occur in the posterior thoracic region. In race III the retarded area is anterior to a much accelerated one in the lumbar region, in race X it is behind an accelerated area in the anterior thoracic region. Race V, however, has a high proportion of normal individuals (with both innominate and brachiocephalics present) as does also race X, but it differs in having a high proportion of innominate rather than brachiocephalic deficient individuals thus indicating acceleration more anteriorly than in race III and more posteriorly than in race X. From this it seems reasonable to conclude that all three of these accelerated growth areas have their limits somewhere in close proximity to the heart. That in race X must have its greatest influence anterior to the level of attachment of the heart. That in race III can have influence very little anterior to the heart and that of race V would appear to extend anteriorly well into the territory of that of race X.

Since the highest peaks of incidence of each variation are not necessarily confined to any one particular mating (generation) such as the F_1 , but may occur in the F_2 or in backcrosses as well, prediction of what will result from any given cross seems to depend entirely upon a knowledge of the relative distribution within the previous parental generation and thus upon the centers of accelerated growth which they define. It seems most probable that the type of aortic arch pattern is not determined so much by the limits of growth areas as by the relative position (adjacent to, at the margin of, or near center of greatest effect) with respect to the important regional growth areas.

Of interest is the fact that the E type of arch, in which the right subclavian artery arises as persistent right dorsal aorta, did not appear anywhere in these crosses, although a very small per cent was observed in race V, and in the selected line of race V this variation did increase slightly. It is also notable that in race IIc as many as 4% of such animals were observed.

This racial difference suggests a hereditary background for such a variation, possibly effective through a rare combination of accelerated regional growth influences. While in this study particular consideration has been given to the relationships of these arch patterns to regional differences in body length, we do not wish to minimize the possible relationships of width, particularly of the thoracic aperture as suggested by Parsons. We believe his idea is of sufficient merit to warrant further study, and genetic material is now available which will make possible a critical examination.

SUMMARY AND CONCLUSIONS

Domestic rabbits can be classified into 20 different types according to the origin and distribution of the major vessels arising from the aortic arch. Matings within and between these types show little indication of dominance and segregation characteristic of mendelian inheritance, although there is a notable tendency for individuals deficient in either the brachiocephalic or innominate arteries to breed true, and for the normal types possessing both of these vessels in well developed form to produce the entire range of variation. Each of three inbred races is characterized by different proportions of these types, and in crosses there is the same tendency for subsequent generations to show a distribution of types intermediate between those of the parent populations concerned. This fact, together with the notable exceptions in which the frequency of certain types exceeds that found in either parent race, and also the fact that in the F_2 and certain backcrosses there is a characteristic increase in the proportion of combination types deficient in both the innominate and brachiocephalic arteries, is considered evidence that these deficiencies are hereditarily determined by their relative position in relation to certain previously demonstrated areas of accelerated growth in this region. The manner in which these variations further serve to define the anterior limits of the different growth centers previously recognized in these races is described.

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CHANGES IN ISLETS OF LANGERHANS IN LIVING MICE AFTER ALLOXAN ADMINISTRATION¹

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FIFTEEN FIGURES

INTRODUCTION

Since the announcement by Dunn, Sheehan and McLetchie in 1943 of their discovery that alloxan monohydrate administered parenterally causes specific destruction of the islet cells of the rabbit pancreas with subsequent changes in the blood sugar levels, the attention of many investigators of carbohydrate metabolism has turned toward an understanding of the specific destructive effect of this chemical on islet tissue. Chemically, alloxan is a ureide of mesoxalic acid and can be formed from oxidation of uric acid with nitric acid.

During the past few years, alloxan diabetes has been produced in various animals, and every phase of this experimental diabetes has been investigated. The pathological sequelae of alloxan administration have been exhaustively studied by numerous investigators but only in stained sections of pancreas removed at various intervals after alloxan injection. It was believed that continuous cytological exami-

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nation of the living pancreas would be more effective. Consequently the technique of Covell ('28) and O'Leary ('30) has been used in this study.

SURVEY OF LITERATURE

In 1862, Liebig reported the detection of alloxan in gelatinous mucus from a case of a patient with intestinal catarrh. Vyvere (1876) claimed to have found alloxantin, a half-reduced product of alloxan, in the intestine of a patient suffering from oxalic acid poisoning. In plants, Lippmann (1896) discovered alloxantin in the hydrolysates from crude beet juice. Fisher and Johnson ('32) also detected alloxantin in bread, (fava) beans and vetch seeds.

Dunn et al. ('43) pointed out that alloxan might be a natural constituent of the body. They thought that, since the main source of uric acid is the muscles, it is possible that alloxan is a natural hormone of muscles, discharged into blood to regulate muscular activity. Because this hypothetical hormone might stimulate the islets of Langerhans of the pancreas, these authors suggested investigation of the lesions in the islets as one line of inquiry into causation of diabetes mellitus. Recent investigations have yielded conflicting results. Karrer et al. ('45) could not find any alloxan-related products in the human body, while Tipson and Ruben ('45) using their pyrrole and modified "purple" tests did detect alloxan in various animal tissues including liver, spleen, brain and thymus. The latter expressed the opinion that overproduction of alloxan, or inability of the body to destroy it, might enable alloxan to reach the pancreas in sufficient concentration to destroy beta cells and thus to produce diabetes.

Archibald ('45) suggested that circulating blood might normally contain a small amount of alloxan in view of the report by Ascoli and Izar ('09) and Preti ('09) of the occurrence in dog's liver, or blood, of an enzyme system capable of splitting uric acid to dialuric acid in the presence of oxygen and of synthesizing uric acid from dialuric acid in

the absence of oxygen. With dialuric acid in the blood, it would not be surprising if minute amounts of alloxan are formed by the action of molecular oxygen carried by hemoglobin.

Investigation of effects of alloxan on animals commenced about 1900. Though Koehne (1894) reported no toxic symptoms in dogs fed with alloxan, Wiener (1899) observed convulsions in rabbits two hours after feeding 0.5 gm of alloxan per kilogram body weight. More recently Jacob ('37) described marked hypoglycemia in rabbits 3–4 hours after alloxan injection, followed by hypoglycemic convulsions and death. Jacob, however, did not examine the viscera histologically and offered no explanation for the phenomena.

Widespread interest was aroused by the very important discovery of selective destruction of islets of Langerhans after alloxan administration by Dunn, Sheehan and McLetchie ('43) already mentioned. At the time, these authors were using uric acid and related products in a study of pathogenesis of renal lesions in the crush syndrome and after mismatched blood transfusions. Among the uric acid derivatives, alloxan seemed significant but it caused death in the first day or so with "distinctive symptoms which were not related to renal disease." It was in the investigation of these early deaths that a lesion was noted in the islets leading to efforts to produce diabetes. Success was reported by Bailey and Bailey ('43) in rabbits, Dunn and McLetchie ('43) in rats, and Goldner and Gomori ('43) in dogs. Since then alloxan had been given to monkeys (Banerjee, '44), pigeons (Goldner and Gomori, '45a), frogs (Seiden, '45), cats (Peralta, '45), ducks and chickens (Mirsky, '45) and owls (Scott, Harris and Chen, '45) with variable results.

MATERIALS AND METHODS

One hundred Swiss mice of both sexes, 15–20 gm in weight and about 6–8 weeks old were employed in these experiments. They were fed Rockland mouse pellets and water *ad libitum*. The mice were injected with 0.3 mg per gram body weight

of a freshly-prepared 2½% alloxan solution in distilled water by three methods. The majority of the mice were injected intraperitoneally; others received subcutaneous or intravenous injections. In order to permit the earliest possible observations and to insure complete absorption of alloxan into the circulation, a few mice, already under observation on the stage of the microscope, were injected in the tail veins. Small skin windows were cut in their tails and no. 27 gauge hypodermic needles were used.

After alloxan administration the general condition of the mice was observed. Urine examinations were made by Benedict's qualitative method but usually not until after the termination of each experiment, when urine was collected from the distended urinary bladders removed *in toto*. Groups of about 10 mice were given alloxan at the same time. At various intervals the mice were anesthetized with veterinary Nembutal (1/50 cm³ per 20 gm weight). The pancreas was exposed according to the method described by Covell ('28) after the skin was shaved on the left side and a small skin incision was made in the abdominal wall overlying the spleen. This incision was carried deeper through the muscular and peritoneal layer exposing the abdominal cavity just under the costal margin. The mouse was then mounted on a specially constructed cork-topped microscope stage. After pulling out the free end of the spleen, the pancreas attached to it was gently spread by camel hair brushes soaked in warm Ringer's solution, and covered by a glass slip having a rounded edge on the side of contact. A mixture of neutral red janus green B, made up in concentration of 1:500 and 1:1000 respectively in normal saline solution, was employed for vital staining of many preparations. This was injected intraperitoneally in the early experiments, but the results proved unsatisfactory owing to precipitation of the dyes, lack of uniformity in staining, and the toxicity of the janus green. Consequently, in the more successful experiments a small piece of gauze was cut, soaked in the mixture and applied to the surface of the pancreas, the mixture was re-

plenished several times. The precipitation was thus avoided. Approximately 10 minutes were allowed for adequate staining. On the average about two hours were required to complete observation of each animal. In a few cases the mice were observed continuously for as long as 10 hours. More studies were made in the first day or two of the experiments than later on since it was found that the islets' changes took place promptly following the alloxan administrations.

About 30 specimens of pancreas were removed after conclusion of the observations *in vivo*. They were fixed in Bouin's fluid, embedded in paraffin, sectioned at 7μ and stained with the hematoxylin and phloxin method of Gomori ('41). This material was used for fixed tissue controls of observations on the living pancreases.

OBSERVATIONS

Diabetic production rate and general conditions

Forty mice were injected intraperitoneally each with 0.3 mg/gm of alloxan to determine the production frequency of alloxan diabetes. Of these 40, one died a day after the injection. Between the third and 7th day, urine sugar was determined in 16 mice. Eight mice showed glycosuria, 5 of them rather severely. Four animals were tested between the 10th and 13th day; only one case of marked glycosuria was found. Examination made after 20 days of the remaining 19 mice showed only three with moderately severe diabetes. Five mice of this latter group were not examined because an insufficient amount of urine was collected.

After administration of alloxan, all mice became quiescent for a time before resumption of normal activity again. The urine was of a marked red color after the first few minutes; but microscopic examination did not reveal the presence of erythrocytes. Most fatalities occurred on the first day after injection subsequent to which the mice regained their healthy appearance and increased in weight rapidly. Two or three

weeks later some mice commenced to lose weight, became emaciated and died. Attempts were not made to save them either by insulin or glucose injections. A few additional mice were given alloxan subcutaneously. These always showed a healed ulcer at the site of injection ranging in diameter from a few millimeters to a centimeter, devoid of hair and covered with a dry scab.

Gross changes

The abdominal blood vessels became engorged within an hour after alloxan administration, and this hyperemia remained for a few days. Pancreatic hyperemia was best seen at low magnification. It was noted that the larger vascular channels appeared thicker and that the capillary loops about the acini stood out more clearly. On the 4th day, many mice showed distended stomachs and intestines. Dryness of the intestinal and pancreatic surfaces, accompanied by body emaciation, was present in a few mice. After 10 days the pancreas lost its usual translucency and appeared dull and white. About 75% of the mice had adhesions between the pancreas and surrounding structures so that it was impossible to mobilize the pancreas for observation under the microscope in the usual manner. Under these conditions the pancreas and spleen adhered firmly to the surfaces of the stomach, intestines and colon. In the more severe cases, the pancreas was attached to the left kidney, liver and the overlying abdominal wall.

Microscopic changes

a. The capsule and its cellular content. As early as an hour after alloxan administration there was marked increase in mononuclear wandering cells inside the connective tissue capsule of the pancreas. The number reached its peak in about 12 hours and stayed at that level with slight fluctuations for approximately 10 days; after that it gradually decreased to the normal level. The number of cells in the

capsules appeared to parallel closely the number of wandering cells in the parenchymatous tissue. In two 12 day specimens, these cells varied considerably in shape and size. Some were twice as large as macrophages. Many had distorted nuclei. In extreme cases, ring-shaped or "moth-eaten" nuclei were present.

The normal islets of control mice had a smooth oval shape and brownish coloration (fig. 1). In animals treated with alloxan, the brownish coloration faded in 10 to 12 hours and the islets appeared pale. The pallor increased in intensity with the interval between injection and observation. In 10 to 12 day specimens the islets became so pale that they were hardly distinguishable from the surrounding acinar tissues. This pallor varied in different parts of the islets. The number of capillary loops was obviously decreased per area of the islet surface. Thus the islets showed diminished vascularity after alloxan treatment, despite the early abdominal vascular engorgement already mentioned.

With intravital staining, changes were even more apparent. The uniformly pinkish-red coloration of the stained normal islet was absent. Within two hours after injection, the cells of the periphery, normally of the alpha cell type, stained a bright red, but the more centrally-placed beta cells stained with janus green B. In 16 hour specimens, while the alpha cell areas were unchanged, the beta cells no longer colored with janus green, remaining pale after contact with the dye. Among beta cells, however, bright red cellular components were appearing (figs. 3 and 4). These were first observed in two hour specimens and rapidly reached the maximum number soon after. Due to accumulation of these cells the islands of Langerhans as a whole had a spotty appearance during the first few days after alloxan administration. After two days, the number of red spots gradually decreased and were almost absent on the 5th and 6th days. These red spots were identified under the oil immersion objective as the macrophages that had infiltrated the islets.

Meanwhile, the shape of the islets was also changing. Whether the mice became diabetic or not, the alloxan-treated islets were slightly loose in their capsules. Among the diabetic mice, a few islets studied were observed to have become very thin (fig. 14), a condition never observed in normal animals. These islets nevertheless continued to stain well with neutral red. The majority of islets in established diabetic cases had irregular outlines and appeared shrunken. In one specimen, the islets had a knotty appearance. These characters could be clearly seen between 5 to 14 days.

The most valuable diagnostic feature was the arrangement of the insular cells. Normally, islet cells arranged themselves in cellular cords with orderly rows of nuclei, separated by woven baskets of sinuous capillaries, with alpha cells arranged in neat groups or rows along the periphery. Beta cells were centrally placed and by far the more numerous. Normal beta cells stain less intensely with neutral red than the alpha cells and the former's granules are finer than the latter's. The occurrence of alpha cells and paler cells in about equal numbers, or the latter even less in number, arranged in a disorderly manner invariably indicated that the specimen belonged to a diabetic mouse.

b. The beta cells. Cytoplasm: islets were under observation under the microscope when alloxan was administered through the tail veins. Observations were thus made possible from the first minute onward. While the earliest changes were so insidious as to make comparisons difficult, it was certain that the first changes in the beta cells occurred in the first half hour after alloxan injection. At 30 minutes (fig. 10), many beta cells, especially the central ones, formed many small fluid vacuoles in their cytoplasm. These soon coalesced to form crescent-shaped vacuoles in the periphery of the cells close to the cell membranes. At one hour, the vacuoles from adjoining cells had joined together; inside the cells they appeared as unstained areas surrounded by the granular cytoplasm. The vacuoles later became so large that part of the fluid escaped from the cells, leaked along intercellular

spaces, and crowded the flanking cells aside, toward the capillaries. There the fluid collected between insular cells and the capillary walls and gradually drained into the vascular channels (fig. 11). The central group of cells or the cells toward the side where the blood vessels entered or left the islets, usually showed this change earlier and more severely. Two hours after alloxan administration almost all the cells formed vacuoles in the cytoplasm (fig. 12). Some cells were filled with fluid, rounded out and dislocated. They appeared under the microscope as perfectly round structures with pale fluid contents. Occasionally, their nuclei were flattened against cell membranes. In some areas the intercellular fluid accumulation had been so great, particularly during the first 8 to 10 hours, that the insular cells were pressed away from the capillaries. The fluid accumulation gradually diminished but was detectable after the first few days in the remaining cells.

Specific beta granules: normally the granules were very fine and uniformly distributed in the cytoplasm of beta cells. With neutral red and janus green in normal saline solution, they took a light red coloration. Half an hour after the intravital staining the neutral red stain was gradually replaced by the janus green. Less than half an hour after intravenous injection of alloxan, when fluid vacuoles were forming at one pole of the beta cells as has been described, there was a proportionate local diminution in the number of beta granules. None were observed to come out of cells into the intercellular spaces. The remaining granules near the vacuoles clumped together (fig. 13). In the other poles of the cells, where there was no vacuole formation, the granules appeared normal both in number and distribution. In two hour specimens, the granules either took the greenish stain of janus dye, or they stain a faint red for a much shorter time than normal. About 8 or 10 hours after alloxan administration, the beta granules failed to take the green dye. While observation at this stage was extremely difficult because of the extensive infiltration with macrophages which

hid most of the beta cells from view, some beta cells appeared very clearly without any granules at all. The majority had colorless coarsely granular cytoplasm with poorly discernible cell membranes.

Nuclei: examination of the nuclei was not entirely satisfactory because they were unstained with vital dyes, and were obscured by the infiltration of large macrophages. Consequently observations were limited to those nuclei that were sufficiently exposed to be examined critically. The earliest changes in beta cell nuclei were observed in about one hour. The nuclei decreased slightly in size though rounded contours were still maintained. From two to three hours, they were definitely distorted with one side or another collapsed and at the same time they appeared paler. Again, as in the case of other cellular changes described, centrally placed cells were more affected than peripheral ones. By 8 to 9 hours, large macrophages obscured the nuclei and their fragments. Only granular debris, unstained by vital dyes, were discernible.

c. The alpha cells. Alpha cells showed no degenerative changes for a long period after alloxan administration. However in 4 to 5 day specimens, they appeared to be proliferating and occupied the area previously taken up by beta cells. Alpha and beta cells were difficult to identify thereafter. There was some indication that cells apparently of the alpha type also underwent degenerative changes similar to those of beta cells.

d. Vascular changes in islands of Langerhans. The blood supply of islands of Langerhans is very rich and the vessels extremely sinuous. Two hours after alloxan injection, the normally flattened endothelial cells appeared slightly swollen. Between two and three hours, large numbers of leucocytes attached themselves to the walls of the capillaries and migrated out into the islet tissue. Blood flow was retarded at about 4 hours by further swelling of endothelial cells. Lumina of the capillaries were restricted so that passages of leucocytes became difficult. Some capillaries appeared to

be blocked as indicated by stoppage of blood flow, presence of a few stationary erythrocytes and little plasma, and by dilation of some capillaries to twice the normal diameter. These apparently obstructed capillaries remained non-functional for three to 4 hours at which time the observations were terminated. It was not at all unusual to see 4 or 5 leucocytes attach themselves to a short section of capillary in a specimen at 10 hours or over. Endothelial cells reached maximum size in about 24 hour specimens. Swellings of endothelial cells were observable as late as 4 days after alloxan administration. The general pattern of the vascular channels of diabetic islets was characterized by a lengthening and straightening of the capillaries.

e. Cell infiltration. The increase in wandering cells in the pancreas was generalized. A large number of cells arranged themselves in neat, long rows parallel to larger blood vessels. The macrophages with very few exceptions could be found either inside or in the vicinity of islets of Langerhans.

One hour after alloxan administration there was a moderate increase in the number of wandering cells. A few large mononuclear cells started to invade the outer rims of the islets. More and more of the cells infiltrated in the next hour. They apparently developed into characteristic macrophages after three hours though none of them was seen outside the islets of Langerhans. At the same time there was increase in lymphocytic cells around the islets. In 18 hour specimens, an islet, about $180 \times 140 \mu$ in size, contained at least 33 macrophages, a great number of medium-sized mononuclear cells and a few small lymphocytes. A few of the medium sized mononuclear cells were found inside the islets at this time.

Macrophages were found overlying degenerating beta cells or adjacent to those that had degenerated into round balls of fluid. They were fairly evenly distributed throughout the damaged areas and in no instances did they invade the still intact groups of alpha cells. Sometimes careful focusing revealed one or two lymphocytes within large collections of

fluid which had escaped from the degenerating beta cells (fig. 15).

These cells were apparently derived from the islet capillaries and from some larger vessels. The latter was indicated by the occurrence of long rows of wandering cells parallel to their courses.

Fibroblasts also showed proliferative changes. In 8 hour specimens large numbers of mitoses were found among them. Various stages in their transformation into medium and finally large mononuclear wandering cells were common. Those nearest the islets developed vacuoles of variable sizes and closely resembled macrophages. Compared to those within the islets, the macrophages outside had fewer and paler neutral red vacuoles. Soon after the invasion of the islets, the macrophages became engorged, and the neutral red vacuoles enlarged and stained a bright red color. Mitoses were evident in the macrophages.

*Pathological changes as seen from
microscopic sections*

Microscopic sections were employed in order to check the accuracy of observations of living preparations and vice versa. Many of the tissues were taken after termination of the experiments.

Sections were stained by Gomori's method. Relatively few beta cells in one hour specimens had slightly shrunken nuclei. Between one to one and one-half hours, nuclei were smaller and some had broken up into homogeneously red-staining pieces. The nuclei of the central cells were particularly affected. A slight decrease in beta granules and vacuoles could be observed in few cells. Cell outlines were still visible. Alpha cells appeared normal. By 4 hours, very few fragments of beta nuclei were left, cell outlines were indistinct and few vacuoles were seen among them. The alpha cells at the periphery still appeared normal, though some apparently contained fewer granules and some cytoplasmic vacuoles.

In 7 hour specimens (fig. 7) beta cells were reduced to granular debris but the normal shape of the islet persisted. The alpha cells multiplied and surrounded the remainder of beta cells. The 9 hour specimens showed evidence of alpha cell destruction. The cords of alpha cells were disrupted, a few of them had clear areas about their nuclei. In the 14 to 15 hour specimens, macrophages containing coarse granules could be identified in the midst of colorless debris. The 16½ hour specimens contained some alpha cells with pale cytoplasm and some vacuoles. Elsewhere in the acinar tissue there were foci of mononuclear cell infiltration. In three to 10 day specimens, there was complete disappearance of beta cells. Alpha cells proliferated and grew centrally (fig. 7). The cellular arrangement was irregular and cells were slightly increased in size. Islets were then composed mostly of alpha cells with few agranular cells (fig. 9). Terminal stages could be seen in 20 day specimens. Many, however, showed complete islet disintegration with only sparse connective tissue frameworks and very few unidentified cells remaining.

DISCUSSION

Diabetes was successfully produced in mice by intraperitoneal injections of alloxan. Symptoms and signs of polyuria, polydipsia, polyphagia and glycosuria were observed. A few alloxan-treated mice died after convulsions, apparently hypoglycemic in nature. After an intraperitoneal dose of 300 mg/kg of alloxan, 50% of the mice examined between the third and 7th days were found to be diabetic. When 19 mice from the same group were tested for glycosuria, 20 days after alloxan treatment, only three gave positive reactions. Since 5 mice of the latter group were not examined because too little urine was collected, it is evident that the number of diabetic mice in the latter group comprised only approximately 21%, which is definitely below the results obtained in the first group. The only plausible explanation for this strange phenomenon is that some mice may have had only

transitory diabetes and their urine may have become sugar-free after a lapse of time. This explanation is in accordance with the report of Carrasco-Formiguera ('44) that 42 days after alloxan administration, dogs that earlier showed glycosuria became sugar-free and had a normal dextrose tolerance. Bailey et al. ('44) injected small amounts of alloxan into rabbits and noted that when injections were discontinued, diabetes became milder.

Alloxan monohydrate is a very toxic chemical. Injected subcutaneously, it caused necrosis and ulceration of the skin. When injected intraperitoneally, alloxan caused vasodilatation of the abdominal viscera, excoriation of hepatic surface, and finally adhesions of internal organs especially near the pancreas. Lieben and Edel ('33) suggested that alloxan was a capillary poison. Duffy ('45) reported irregular dilatation of the aorta of the treated rabbits with roughening of the lining from the valve to the bifurcation. There were fatty changes in the intima.

With diabetic doses of alloxan which varied in different animals (Goldner and Gomori, '44), the only lesions found in the body were in the islets of Langerhans, with symptoms comparable to diabetes mellitus. Overdosage also produced degenerations in the kidneys, adrenal, liver and pituitary (Dunn et al., '43; Bailey et al., '44; Goldner and Gomori, '44 and '45; Hard and Carr, '44; Duffy, '45; Kendall et al., '45). Ruben and Yardumian ('46) fed cats with alloxan. Besides having marked albuminuria, 4 of the 10 cats developed upper respiratory irritations with sneezings and frothy mucus in the external nares. There was one cat with conjunctivitis and one with bloody stools. Brunschwig et al. ('44) treated a patient suffering from inoperable metastasized islet cell carcinoma of the head of pancreas with alloxan. They noticed definite improvement in the general condition of the patient. However, toxic symptoms were observed such as nausea, emesis, coma, icterus and depression. A chill occurred with subsequent rise in temperature. The erythrocyte count was

diminished. A burning sensation was experienced in the epigastrium.

Injury to the beta cells by alloxan is manifested in less than 30 minutes. The first changes seen were fluid vacuole formation in the cytoplasm with local disappearance of beta granules. Since the diminution of the beta granules was noticed to be proportionate to the increase in size of the fluid vacuoles, and because no granules were seen to escape from the cells, it appears that the vacuoles are produced by liquefaction of the granules. The process of vacuolation continued for about 12 hours and represented the period of hydropic degeneration. Fluid gradually escaped from the degenerating cells and drained into the capillaries. The granules finally disappeared completely from the beta cells. In addition, the beta granules, instead of taking the red stain of the normal beta cells, took either the greenish coloration of janus green or remained unstained. In the control specimens, the beta granules would stain green from 30 minutes to one hour after application of the dyes which was apparently due to a lack of oxygen. Consequently, the beta cells also showed a possible early oxygen insufficiency.

The granular debris, seen after 12 hours, was not made up of zymogen granules, since the former were large and glassy compared to the latter's small and opaque characters. They were possibly the broken down cytoplasm.

Within a period of three hours, the beta cell nuclei became smaller and distorted. At about 8 to 9 hours, they fragmented in the cytoplasm and evidently were removed by the invading macrophages.

Many investigators including Goldner and Gomori ('44), Ruben and Yardumian ('45) reported that they could not find inflammatory cells during the period of degeneration of the islets. Bailey, Bailey and Hagan ('44) noticed no infiltration of polymorphs or wandering cells. In the present study mononuclear cells migrated out of the blood circulation. Others were transformed from connective tissue fibroblasts as many intermediate stages between the two were frequently

observed. Multiplication by mitosis was also noticed. Inside and around the islets, the mononuclear cells enlarged, developed many neutral red cytoplasmic vacuoles, and finally took the form of typical macrophages. The macrophages inside the islets were observed to be richer in neutral red vacuoles than those outside and the vacuoles stained a darker red. These macrophages were at a maximum up to the second day at which time they gradually decreased in number. In general, the repair of the islets was complete after 5 to 6 days though the islets were left deformed.

Investigators, among whom were Goldner and Gomori ('44) and Duffy ('45) believed that alpha cells were not affected by the alloxan administration. However, Bailey, Bailey and Leech ('46) observed that alpha cells increased in number, had pyknotic nuclei and agranular cytoplasm at the stage they studied. Duff and Starr ('44) also noticed that some centrally-placed alpha cells were necrotic. But since Dunn et al. ('43), Goldner and Gomori ('44) and Bailey, Bailey and Leech ('46) believed that many islets had completely disappeared, the hypothesis that the alpha cells were also destroyed seemed substantiated. In this investigation it was observed, in both the living preparations and in the stained sections, that alpha cells were also degenerating though much more slowly than the beta cells.

Changes in the blood capillaries of the islands of Langerhans were also evident. Endothelial cells enlarged two to three hours after alloxan injection, and capillary walls became sticky. The blood flow in many capillaries decreased in amount and finally stopped. The stoppage was apparently a result of a collapse of the capillary wall by pressure from the fluid escaping from the degenerating beta cells, the marked swelling of the endothelial cells, and the attachment of numerous leucocytes to the capillary walls.

Stained preparations can be studied at leisure and structures can be sharply outlined. But the examination of living tissues, while less clear, makes possible dynamic and progressive observations. While many investigators have studied

many pathological sections of the alloxan-treated pancreas, only Ridout et al. ('44) found that many of the regenerated cells at 48 hours were "fibroblasts." This was the only previous account of wandering cells in alloxan-treated islets. However those "fibroblasts" apparently were not regenerated islet cells but the mononuclear cells infiltrated into the islets.

Hughes et al. ('44) and Ridout et al. ('44) reported widening of the pericapillary spaces; but no reference was made by them to the possibility that the widening might be due to an accumulation of fluid from the degenerating beta cells.

The present study indicates that Gomori's differential staining method does not permit identification of macrophages. This would explain the failure of previous investigators to recognize them. It should be noted that Hughes et al. ('44) stated that some alpha cells contained eosinophile granules.

CONCLUSION AND SUMMARY

1. Alloxan diabetes was successfully produced in mice but in some this was only transitory.

2. Alloxan is a local irritant. It produced necrosis and ulceration of skin when injected subcutaneously. Given intraperitoneally it produced irritation of abdominal viscera leading to peritoneal adhesions.

3. Less than 30 minutes after alloxan administration, fluid was accumulating in the cytoplasm of the beta cells with a corresponding decrease in the number of granules. Later, the fluid escaped from the cells and collected beside the capillaries, whence it drained.

4. The earliest changes in the granules included fading, clumping, and changes in staining properties suggesting decrease in cellular oxygenation.

5. The beta cell nuclei became small and fragmented.

6. Marked cell infiltrations of the mononuclear types were observed. The most notable were the macrophages that developed from the smaller mononuclear cells and the fibroblasts.

7. Contrary to general belief, the alpha cells were found to be degenerating though much more slowly than the beta cells.

8. The endothelial cells of the capillaries were swollen. This, with the inward pushing of the capillary walls by fluid escaped from the degenerating beta cells and the attachment of a large number of leucocytes to the walls forming a thrombus, tended to impair the blood flow. Many capillaries were functionless and disappeared.

9. The diabetic islet was pale. Its capillaries were fewer in number, longer and straighter. The islet itself appeared collapsed and distorted. Irregularly arranged alpha cells predominated.

10. The relative merits of living and stained section study are discussed.

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PLATE 1

EXPLANATION OF FIGURES

1 Normal control islet has an oval shape. It has a smooth outline and stains uniformly. The evenly-spaced insular cells have finely granular cytoplasm and round or oval nuclei. $\times 1800$.

2 Five hours and 15 minutes after alloxan injection. Beta cells stain poorly in contrast with normally stained alpha cells. $\times 500$.

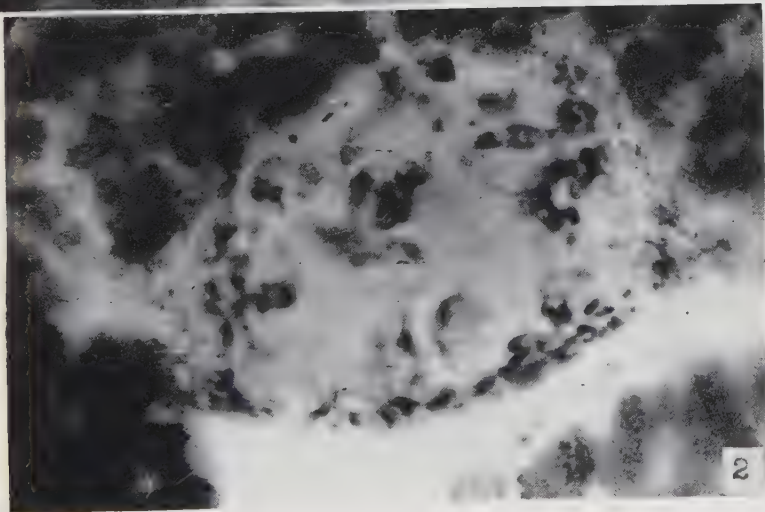
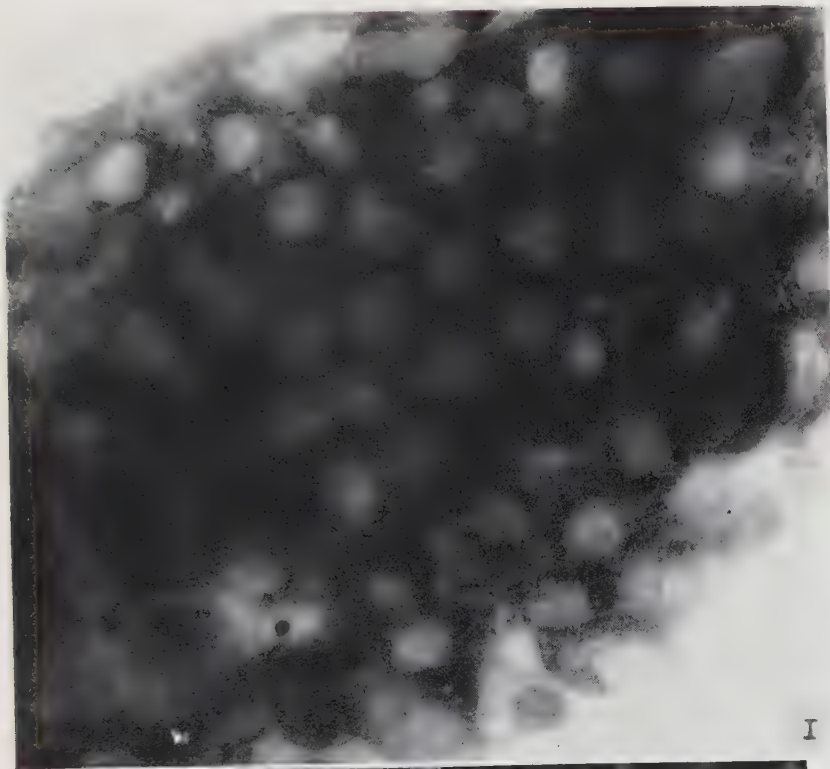


PLATE 2

EXPLANATION OF FIGURES

- 3 Twelve hour specimen. The islet appeared spotty, having a large number of red spots against the pale background. $\times 500$.
- 4 Same specimen with oil immersion. Macrophages can be identified clearly. Areas of intact alpha cells were not invaded by the macrophages. $\times 1200$.
- 5 Twenty-four hour specimen. Showing similar changes with figures 3 and 4. The islet structure appears collapsed slightly. $\times 500$.

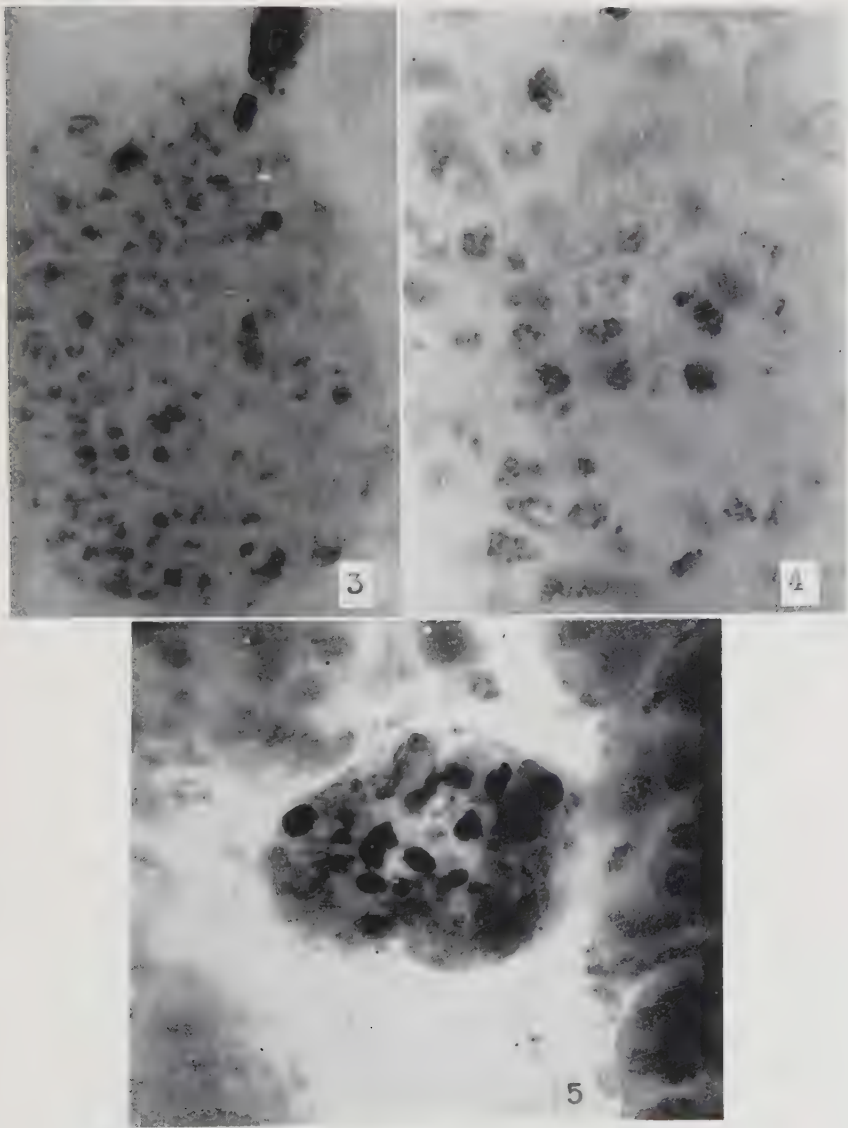


PLATE 3

EXPLANATION OF FIGURES

6 Two and one-half hours. Advance pyknosis of beta cell nuclei. The nuclei are distorted, with small fragments cast off. Many vacuoles are visible in the preparation. (See arrows.) The alpha cells form a distinct group at the periphery.

7 Seven and one-half hours. Beta cells in the middle of the islet are completely destroyed though the general form of the islet is intact. The group of alpha cells at one side of the islet appear normal.

(The above figures are stained by Gormi's hematoxylin and phloxin stain.)

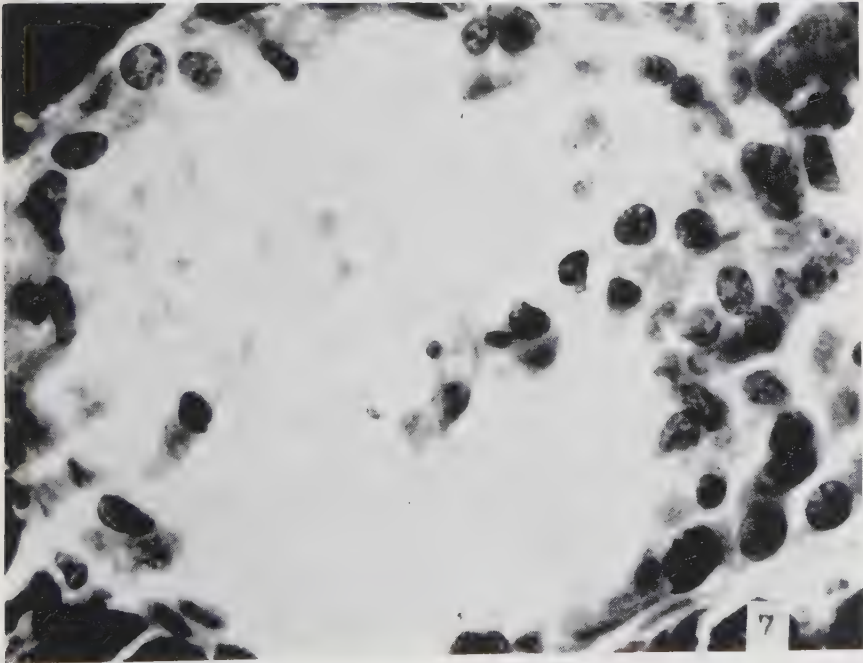
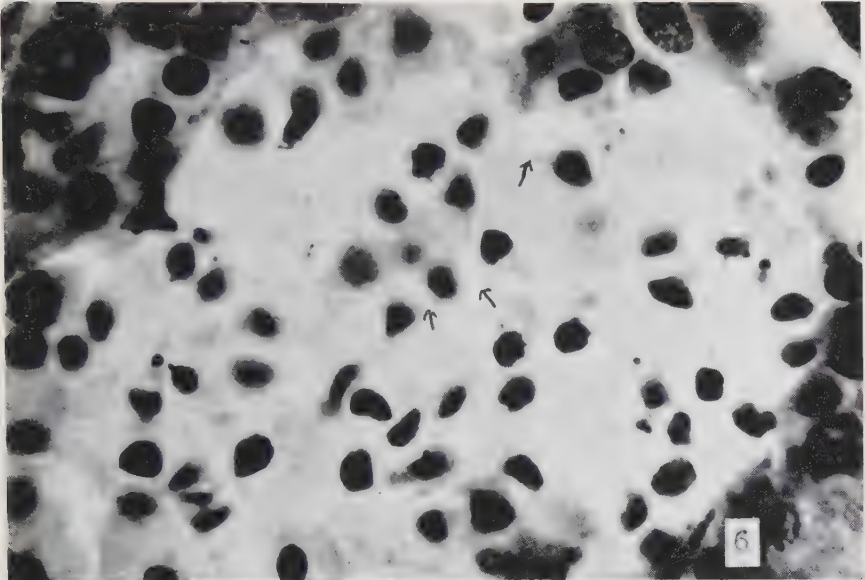


PLATE 4

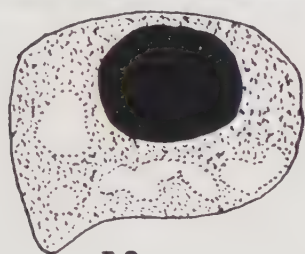
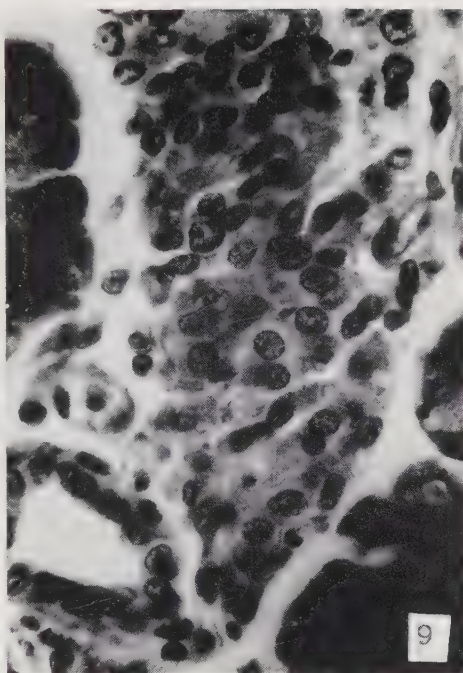
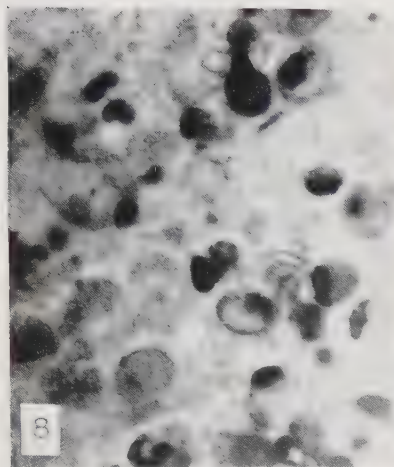
EXPLANATION OF FIGURES

8 Sixteen and one-half hour specimens showing large number of macrophages.

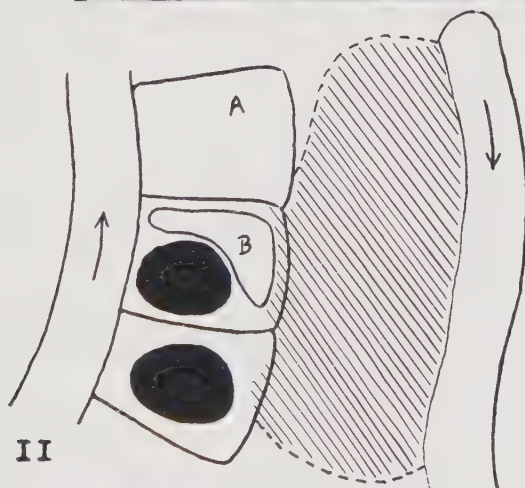
9 Islet of a diabetic mouse (uring sugar + + + +). Five day 19 hour specimen. Notice the distorted shape of the islet. The islet is long and thin. Alpha cells are crowded together.

10 Drawing of one cell from one and one-half to two hour specimen, showing the fluid vacuoles in the cytoplasm.

11 Thirty minutes after alloxan. An area between two capillaries. Arrows show the directions of the blood flow. "A" cell is filled with fluid. "B" is a vacuole in the cytoplasm of another cell. The third cell is unchanged. The shaded area represents fluid collection outside the cells obscuring the islet cells underneath it.



10



11

PLATE 5

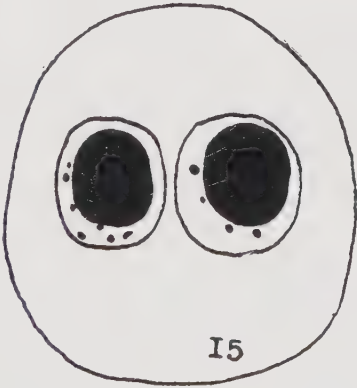
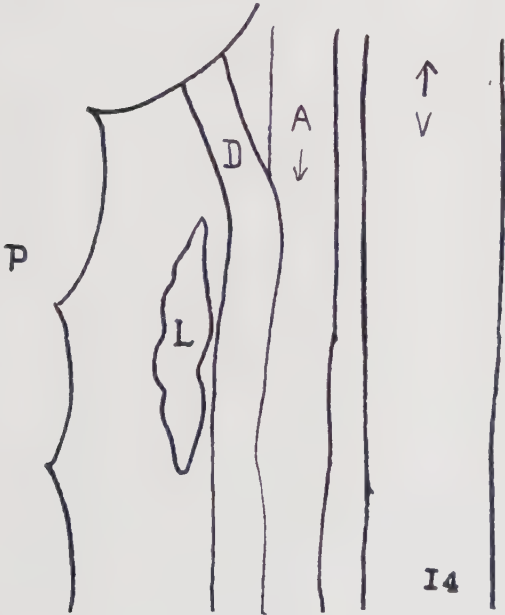
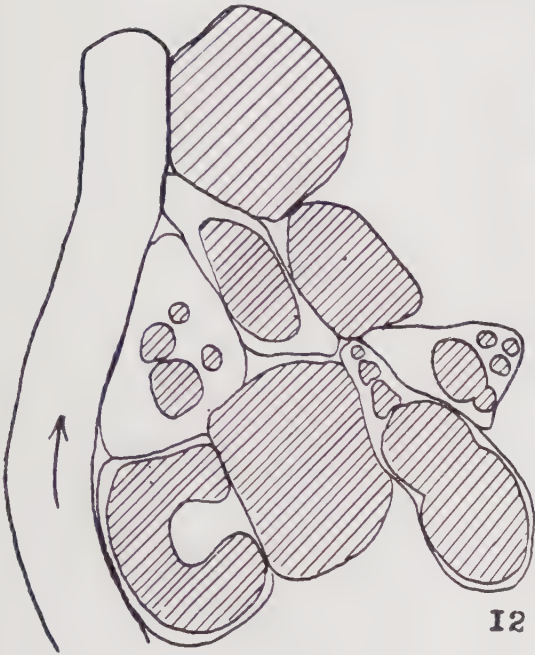
EXPLANATION OF FIGURES

12 Four to 5 hours. Diagrammatic drawing of a group of cells. Cells are greatly swollen. There are variable numbers of fluid vacuoles (shaded areas) inside the cells.

13 One hour specimen. One islet cell has clumps of granules separated by fluid in the cytoplasm. The other cell has similar changes on one side of the cell only. The other half is apparently normal. The fluids in the two cells have coalesced.

14 Diagram of a three day 17 hour specimen. A, artery; D, interlobular pancreatic duct; I, islet of Langerhans; P, pancreatic acini; V, vein. Notice the collapsed and irregular appearance of a diabetic islet. Urine sugar + + + +.

15 Drawing made from 29 hour specimen showing a large round collection of fluid between two loops of capillaries and two lymphocytic cells with few neutral red granules. Careful focusing revealed that the cells were inside the fluid globule.



THE STABILITY OF HYDROGEN-ION CONCENTRATION IN FIXATIVES ¹

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The full role of hydrogen-ion concentration in the numerous fixatives used in histological technique is not fully understood but the evidence indicates it may play an important part in the phenomenon of coagulation of proteins with which most fixing mixtures are concerned. Its significance seems to lie in the fact that proteins undergo maximum precipitation from solutions with the same pH as their isoelectric points because the colloid particles are electrically neutral. Also, proteins are known to be amphoteric in nature so they unite with kations on the alkaline side and with anions on the acid side of their isoelectric point.

The present investigation is concerned with a determination of the pH and its stability in 50 commonly used fixatives in histological technique. The formulas were selected from the latest editions of Lee's "Vade Mecum," McClung's "Microscopical Technique," Mallory's "Pathological Technique" and also certain issues of "Stain Technology." Some fixing mixtures penetrate the tissues very slowly so that the fixation time is prolonged whereas others are known to undergo decomposition in relatively short periods necessitating frequent changes. It was deemed worth while to see if and how the pH of fixation might change on standing under ordinary circumstances. The results will also include hydro-

¹ Aided by a grant from the Committee on Scientific Investigation of the American Medical Association.

gen-ion concentration values of a number of fixatives not already recorded in the literature.

Table 1 gives a list of the 50 stable and unstable fixatives tested as to hydrogen-ion concentration with a Beckman pH meter, Model H-2. It shows first that 47 out of the 50 are within the acid range. This agrees with the general findings of Petrunkevitch and Pickford ('36) who tested a smaller series, some of which we have repeated.

The pH does not undergo a change on standing in 20 of the fixatives whereas in the remaining 30 it does in minor or major degree. The perfectly stable solutions include such common fixatives as Bouin, Hermann, Erlicki, Champy and osmic acid.

The hydrogen-ion values in the unstable group may change up to about 130% of that obtained in the fresh condition. In most, the shift is toward the alkaline side (23 out of 30 cases). In this series, are such fixing mixtures as Carnoy-Lebrun, Helly, Marina, Maximow, Orth and Regaud. Solutions containing such oxidizers as chromic acid, mercuric chloride and potassium bichromate seem to have the greatest instability of hydrogen-ion concentration. In some instances, the alterations in pH can be correlated with visual signs of decomposition in the mixtures such as precipitation, turbidity or color changes. This is not always true, however, as a few remain clear while pH values are changing particularly in the group having chromic acid. All of the unstable pH mixtures possessing potassium bichromate have a precipitate formed after standing. The speed of the pH change, when it occurs, is almost always gradual rather than immediate. It is believed that the determination of these mutations in hydrogen-ion concentration in fixatives constitutes a delicate method for detecting instability of the chemical reaction.

In conclusion, the results seem to indicate that almost all fixatives function well within the acid range probably because of the fact that the primary fixing solutions are largely acid in nature (unpublished data) and because on mixing they may follow certain laws of hydrogen-ion concentration. Secondly,

Records the stability of pH values and ingredients in 50 commonly used fixative mixtures

[illegible]

about 60% of the fixatives studied in the present series show an instability in pH of minor or major degree on standing which may have significance in the fixation process. Finally, it is believed that determination of changes in hydrogen-ion concentration may constitute a delicate test for detecting signs of chemical decomposition in fixing mixtures.

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- PETRUNKEVITCH, A., AND GRACE E. PICKFORD 1936 On the relative acidity of histological fixing fluids. *Anat. Rec.*, 65: 461-466.

DEMONSTRATION OF BLOOD VESSELS AND LYMPHATICS WITH A FLUORESCENT DYE IN ULTRAVIOLET LIGHT ¹

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SEVEN FIGURES

The present study is an attempt to work out a new method for demonstration of both the distribution and the functional status of the blood vessels by means of an injection method. The principle of the method is the injection into the bloodstream of a vital dye which will be taken up by the walls of the vessels. In this way only the blood vessels actively functioning at the time of injection are stained and rendered visible in histological sections.

METHOD

The fluorescent dye,³ thioflavin-S (methyldehydrothio-p-toluidine-sulfonate) was selected for this study because it stains the vessel walls without injuring them. It is a yellow dye, soluble in water and non-toxic in the quantities necessary for these experiments. No other fluorescent dyes have been found which stain the vessels in this way. Fluorescein, thioflavin-TG, fluodyne, anthracene and phosphin have been tried but none of them have proved to be of practical value, because they diffuse out of the blood vessels almost simultaneously with the injection.

¹ Presented at the sixty-second meeting of the American Association of Anatomists in Philadelphia, April 1949.

² Fellow of the Rockefeller Foundation.

³ Obtained from Hartmann-Leddon Co., Philadelphia, Pa.

Observations were made with mice, guinea pigs, rabbits, and rhesus monkeys. One to three cubic centimeters per kilogram of body weight of a saturated solution of thioflavin-S in water was injected into a vein. The tissues to be studied were instantly frozen in situ with isopentane chilled with liquid nitrogen according to Gersh's ('48) technic. The tissues were removed, cut in thin blocks by a small circular saw, dehydrated at a temperature of about -40°C . and embedded in paraffin. Sections were cut at 25μ , cleared in xylol and mounted in Canada balsam. Water was not used for stretching the sections after cutting because it causes the dye to diffuse out of the blood vessels into the tissues. When necessary, the sections were stretched manually.

Microscopic examination of these sections was made with reflected ultraviolet light. The blood vessels appear as shining channels (figs. 1, 2, and 3), whether or not they contain blood, as long as blood was flowing through them at the time of injection. The length of time between the injection and the fixation of the organ seems to be of utmost importance in order to limit the distribution of the dye to the blood vessels only.

Observations were made on tissues in situ in living animals also, in order to follow the fate of injected thioflavin-S. With this procedure blood vessels were studied in the rabbit's ear, in the mesentery of both the rabbit and guinea pig, in the intestines of guinea pigs and in transparent chambers in mice (Algire, '43).

OBSERVATIONS

When the blood vessels of the living animal are examined in visible light (as opposed to ultraviolet light) thioflavin-S injected intravenously has no effect. When the blood vessels, injected with the dye, are examined in ultraviolet light, however, a violent vasoconstriction is observed (Schlegel and Algire, '49).

When the blood vessels are observed in ultraviolet light during the injection of thioflavin-S, the dye first makes the

arteries visible, then the capillaries and finally the veins. The dye first accumulates in the walls of the vessels. After one to two minutes it begins to leave the vessels and diffuses out into the tissues. After this has taken place the blood vessels appear black against the bright fluorescent tissue. Somewhat later the dye is taken up by the lymphatics, making these vessels fluoresce (figs. 4 and 5). They become, in time, by far the most brilliant structures in the field, as the lymphatic endothelium takes up the dye selectively. In this way, the valves in collecting lymphatics have been seen clearly (see fig. 5). In pieces of excised mesentery, fixed in absolute alcohol for 24 hours and cleared in xylol, the lymphatics are seen perfectly outlined.

The best result with fixed slices after injection of the blood vessels is obtained if the organ is frozen within one or two minutes. After this interval all the blood vessels which are actively functioning at the moment of injection have their walls stained. Artificial shrinkage of the vessels does not detract from the value of the result because their stained walls can still be seen. If for instance arteriovenous anastomoses have been open, it is possible to trace them. Ischemic tissue can be recognized by the lack of staining of the blood vessels, hyperemic tissue by brilliant staining.

The organ mainly used in the study of fixed slices has been the uterus. The purpose was to investigate the occurrence of arteriovenous anastomoses in the endometrium of the rhesus monkey. These structures have been demonstrated in the human endometrium (Schlegel, '45, '46). It is planned to study this problem further in monkeys, and to determine the function of these structures so as to ascertain their possible role in the mechanism of menstruation. So far, attention has been concentrated on the development of the technic but some injections of monkey uteri have been made (figs. 2, 6, and 7). Since thioflavin-S is soluble in water, counterstains such as thionin dissolve the thioflavin-S, thus making the tissues useless for further study in ultraviolet light. The slides from

the rhesus uteri used in this study, therefore, have been photographed in ultraviolet light and then stained with an aqueous solution of thionin. By examining the counterstained slides for histological details and comparing them with the corresponding photographs made with ultraviolet light it is possible to identify direct connection between arterioles and venules. Not enough material is available to give a complete answer, but it appears reasonably certain that the endometrium of the rhesus monkey contains arteriovenous anastomoses (fig. 7).

Although interest has been centered on the blood vessels of the uterus, enough observations have been made on the lymphatic vessels to make us believe that the method would be of especial value in studying this system. Also the rate of absorption by lymphatics can be determined if the interval between the injection and the fixation is varied.

DISCUSSION

To study the blood vessels in internal organs is a rather difficult task. It becomes especially complicated when the vascular bed is surrounded by thick muscular tissue, as in the case of the endometrial blood vessels.

The task becomes even more complex when we intend not only to study the morphology of the blood vessels, but also the distribution of blood flow at the time of injection.

The quartz rod technic, described by Knisely ('36) can hardly be applied to the endometrial vessels, unless the myometrium can be removed. This doubtless would cause unphysiological conditions in the vascular bed.

The method described by Markee ('38), in which pieces of endometrium of the rhesus monkey are transplanted to the anterior chamber in the eye, is extremely valuable. Still it cannot be claimed to be quite physiological, as the tissue is removed from its natural surroundings and vascular connections.

The method described in this paper has the advantage of giving a morphological as well as a physiological picture of

the state of the vessels at the time of injection. Even if the fixation of the tissue causes shrinkage, it is still possible to trace actively functioning vessels, as the injected dye is taken up by the walls of the vessels when blood is flowing through them. The fact that the thioflavin-S is located in the walls of the blood vessels, when the tissue is fixed one to two minutes after the intravenous injection, seems to be related to its rate of diffusion rather than to any specific preference for endothelium or muscle cells. The difference between thioflavin-S and other fluorescent dyes seems to be a difference in the speed with which the dyes diffuse out of the blood vessels. While thioflavin-S requires a considerable length of time in order to diffuse through the walls of the blood vessels, the other fluorescent dyes tried in these experiments diffuse out into the surrounding tissue almost simultaneously with the injection. The matter of timing thus becomes of uttermost importance.

The use of thioflavin-S by intravenous injection for the purpose of vital microscopic studies in ultraviolet light presents, however, certain complications as the thioflavin-S is a powerful photosensitizer. The dye in itself has no effect on the vessels and is not toxic, but if light, especially with a wave length around 3600-3800 Å, is applied when the dye is within the vessels, an unphysiological reaction will occur of sustained vasoconstriction (Schlegel and Algire, '49).

The author wishes to express his gratitude for the valuable assistance of Mr. Chester F. Reather, who has taken all the photographs, and of Mr. J. P. Drane for preparations of the tissues for histological examination.

SUMMARY

Blood vessels were selectively stained by means of intravenous injection of a saturated solution of the fluorescent dye, thioflavin-S. The dye was non-toxic in the dosage used and caused no damage to the blood vessels.

Tissues fixed according to the Gersh freezing-dehydration technic were examined with a microscope in ultraviolet light.

All the blood vessels which were actively functioning at the time of injection could be seen whether or not they contained blood at the time of injection.

Examinations in living animals revealed that the dye diffuses slowly through the walls of the vessels. The dye will be in the walls of the blood vessels if the specimen is fixed one to two minutes after the injection. If the tissue is fixed three minutes or more after the injection, the lymphatics will have taken up the dye, and appear as clearly illuminated channels in ultraviolet light but the blood vessels will no longer be stained by the dye.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

All figures are photomicrographs taken with ultraviolet light.

- 1 Blood vessels of the uterus of a rabbit injected intravenously with thioflavin-S. $\times 100$.
- 2 Blood vessels of the uterus of a rhesus monkey injected intravenously with thioflavin-S. $\times 60$.
- 3 Blood vessels from the intestine of a rabbit injected intravenously with thioflavin-S. $\times 125$.

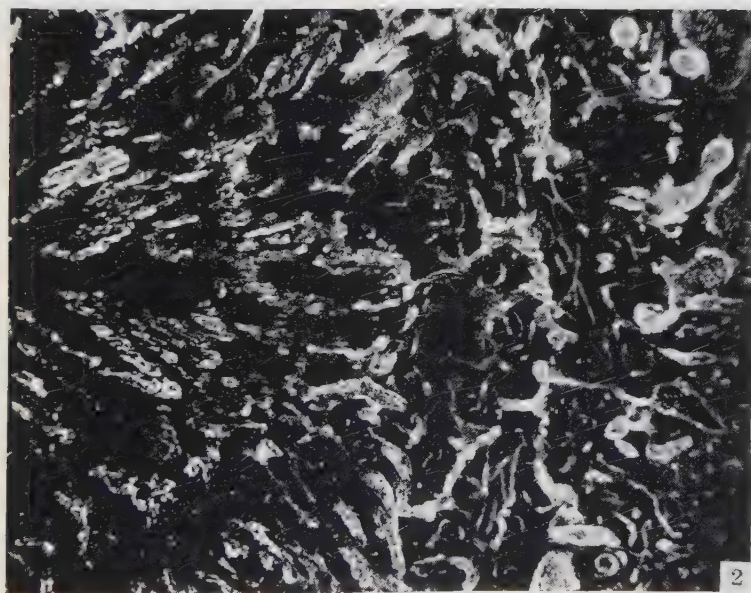


PLATE 2

EXPLANATION OF FIGURES

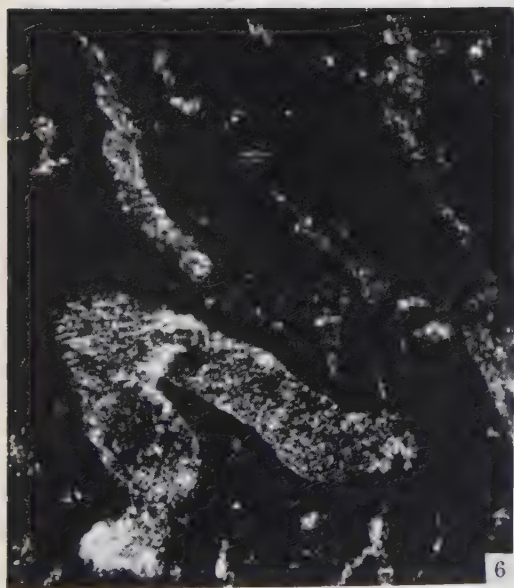
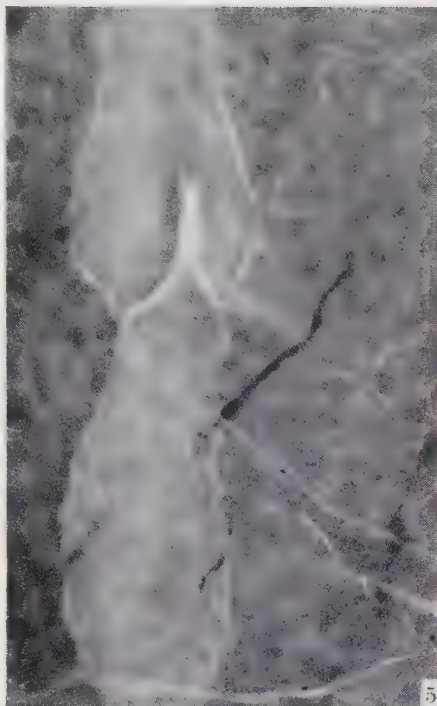
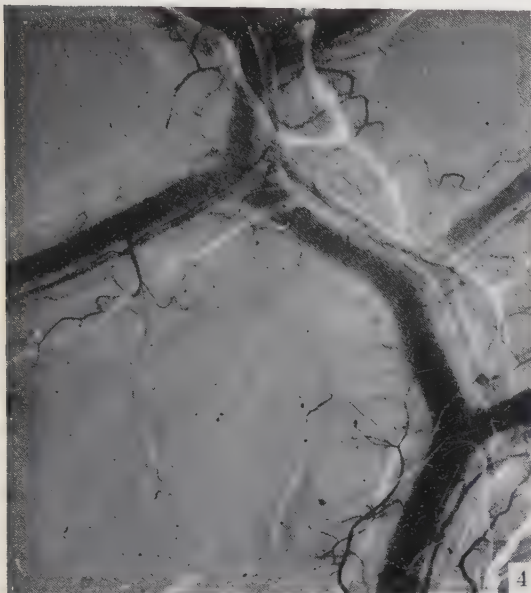
All figures are photomicrographs taken with ultraviolet light.

4 Lymph vessels from the mesentery of a guinea pig injected intravenously with thioflavin-S. The lymphatics appear white. $\times 10$.

5 Lymph vessel showing valves from the mesentery of a guinea pig injected as in figure 4. $\times 100$.

6 Blood vessels in the endometrium from a rhesus monkey injected intravenously with thioflavin-S. Note the large venous lake. $\times 125$.

7 Same as in figure 6. Arrow indicates possible arteriovenous anastomosis. $\times 125$.



RENAL AGENESIS IN THE FEMALE CAT

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TWO FIGURES

During the dissection of a female cat, an anomalous condition of the urogenital system was discovered. The left side of the system was almost normal, whereas the right side showed complete renal agenesis and absence of the uterus and uterine tube. An examination of the literature failed to reveal a single case that was identically the same.

DESCRIPTION

The left kidney measures 3.38 cm by 2.05 cm by 1.84 cm which is slightly larger than one finds in normal cats of this size. In the latter, the corresponding dimensions are 2.93 cm by 1.80 cm by 1.60 cm. The right kidney is absent (fig. 1).

The left ureter is normal in position and opens into the left side of the base of the bladder along the vesico-uterine fold. The complete absence of even a stump of the right ureter was demonstrated by blocking off the left ureter and vagina. The urinary bladder was then injected with dilute India ink. As a result the left ureter became partially filled, but there was no trace of a right ureter or of a right orifice in the bladder. The bladder appears smaller than in a normal cat.

The left suprarenal gland is normal in position and is supplied by a suprarenal artery. The right suprarenal gland is present in its normal position. In the region of the kidney an artery passes laterally from the right side of the abdominal

aorta to the body wall thereafter supplying the suprarenal gland and ovary. The right suprarenal artery apparently had supplied the right suprarenal gland, but its connection with the latter was not very clear. A vein at the level of the



Fig. 1 Ventral view of cat showing unilateral renal agenesis.

kidney leaves from the dorsal rather than from the lateral side of the vena cava inferior and is distributed to the body wall of the right side. This vein branches anteriorly to care for the suprarenal gland and also passes posterior to the ovary.

The left ovary appears to be underdeveloped, but the oviduct is slightly larger than in a normal cat. The left ostium tubae is in the usual position with respect to the ovary. The right ovary is slightly smaller than the left ovary. The

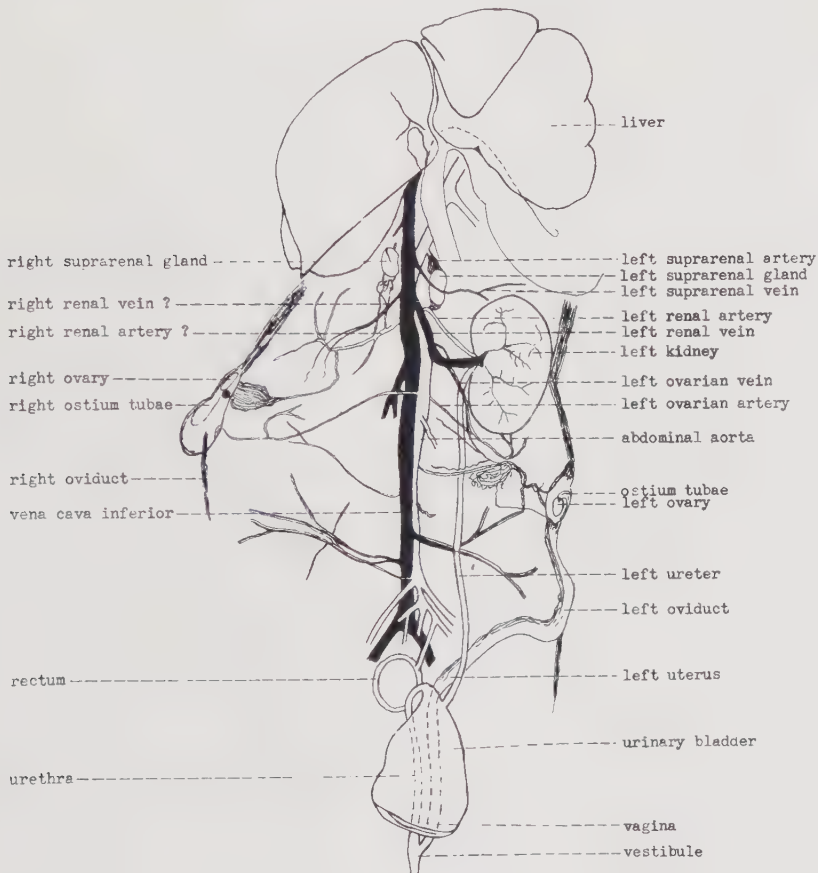


Fig. 2 Drawing made from the cat pictured in figure 1.

ovaries appear in the ordinary position. The right ostium tubae is posterior to the right ovary instead of lateral to it. The tube is small and ends abruptly after extending caudally about one and one-half inches.

The vagina is narrow and lies completely on the left side. There is only a left horn of the uterus. The body of the uterus also lies on the left side as mentioned above for the vagina.

A right arterial branch, which appears to be in the position of the renal artery, extends laterally from the abdominal aorta to the body wall (fig. 2). This branch subdivides to form the above mentioned anterior vessel leading to the suprarenal gland, and a posterior one leading to the right ovary. Both ovarian arteries are smaller than in a normal cat of the same size. The vena cava inferior receives a dorsal vessel similar to a renal vein in position and having tributaries from the right body wall, the suprarenal gland, and the ovary.

DISCUSSION

An attempt was made to determine the approximate age of the embryo when the defect arose. The clues are the presence of the right ovary with a proximal part of a Müllerian duct, and the absence of the right kidney and ureter. The anterior growth of the ureter and the posterior growth of the oviduct have both suffered interference. According to Minot's ('10) discussion of the urogenital ducts, a single duct is primitively formed for each urogenital ridge. This duct is commonly known as the Wolffian duct and becomes the ureter in the female. The second canal known as the Müllerian duct appears a little later in the history of the embryo and closely parallels the first, but has no connection with any of the excretory apparatus and later serves as the female genital duct. The uterine tubes, the uterus, and the vagina are developed from the Müllerian duct.

Hunt ('18) and Tannreuther ('23) have reported cases similar to ours except that their cats were males. The right ureter in Tannreuther's cat was missing, but was partially present in Hunt's cat. Tannreuther studied slides of the 10 mm cat embryo in which he found that the Wolffian duct at this stage was attached to the cloaca.

In Keibel and Mall's Manual of Human Embryology there is a statement by Felix that in man: "At the very time when the posterior end of the groove is separating from the epithelium it begins to grow out caudally and in this process we come to the development of the distal portion of the Müllerian duct. It is formed by the gradual outgrowth of the tip of the cornu."

Minot ('05) has studied the rabbit embryo and concludes that "the ureter appears at $11\frac{1}{2}$ days in a 6 mm embryo rabbit, and at 15 days the Müllerian ducts are present lying on the coelomic side of the Wolffian ducts, extending only a short distance and opening into the coelom at the cephalic part of the Wolffian body."

Pohlman ('04) studied the embryos of Mall and concluded that the abnormalities of the ureter are dependent on the renal bud. The anomalous kidney seems to result from an abnormal development of the renal mesenchyme which appears to be influenced by the renal bud.

CONCLUSION

Considering the evidence gathered from these sources it is here suggested that in our particular cat a disturbance arose early enough to suppress the ureter. Radasch ('08) in reviewing the literature for human and cat anomalies of this sort, concluded that the loss of the kidney is either due to the failure of the ureteric evagination or its retrogression after its appearance. Tannreuther found that the Wolffian duct becomes attached to the cloaca as early as the 10 mm stage. Because our cat completely lacked the Wolffian duct and perhaps the Müllerian duct as well it would seem that the right urogenital ridge was suppressed completely prior to the 10 mm stage preventing the formation of these ducts. Because the early embryology of the cat has been so little studied, specific data appears to be wanting that would enable us to state the exact stage at which interference occurred.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Russell L. Mixter and Mr. Cyril E. Luckman for suggestions and criticisms, and Mr. Malcolm D. Winter, Jr., for preparing the photograph.

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PROGRAM FOR THE FORTY-SIXTH ANNUAL
MEETING OF THE AMERICAN SOCIETY
OF ZOOLOGISTS

DECEMBER 28, 29, 30, 1949

The Society will meet in conjunction with Section F of The American Association for the Advancement of Science, and in association with other biological societies. Most meetings for the presentation of papers and all symposia will be held in the Statler Hotel near the Pennsylvania Station in New York City. The general demonstration program will be held in the biological laboratory building, Schermerhorn Hall, and the demonstrations by motion pictures in Fayerweather Hall on the campus of Columbia University just opposite Schermerhorn.

As features of the meetings, special sessions have been arranged. Dr. Robert Chambers is organizing the presidential symposium on "Experimental Cell Research." A joint session will be held with the Ecological Society of America on the subject "Animal Biology and Sociobiology" which is being arranged by Dr. J. P. Scott. Dr. J. H. Bodine, in cooperation with Dr. L. V. Domm, is organizing a symposium on "Steroid Hormones and Sex Differentiation in Vertebrates" as the A.A.A.S. zoological symposium which will be sponsored by our society.

The annual dinner of The American Society of Zoologists, to which all zoologists and friends are invited, will be held at 6:00 P.M. on the first evening of our meetings, Wednesday, December 28th, in the combined Gold and Oak rooms of Hotel Martinique. Dr. Harley J. VanCleave, Vice-President of The American Association for the Advancement of Science and Chairman of Section F, will deliver the annual address on

"The Development of a Research Interest." Reservations for the dinner should be made not later than Wednesday noon.

The Biologists' smoker will be held on the second evening of the meetings, Thursday, December 29th, at 9:00 P.M., at The American Museum of Natural History.

Members are urged to register with the A.A.A.S. at the registration desk in the Statler Hotel as soon as they arrive in New York if they have not already done so by mail. The expense of projection equipment and operators will be very high in New York, and the amount that the A.A.A.S. will pay toward this expense will depend on the number of our members who register.

The secretary has received no notification of special reduction in railroad fares for the New York meeting. However, members are advised to consult their local railroad agents in regard to possible convention or holiday rates.

As in previous years, the Society is highly indebted to The Wistar Institute of Anatomy and Biology for the prompt publication and mailing of this program.

LOCATION OF MEETINGS

The Statler Hotel is the headquarters of the Society where the symposia and reading of most papers will take place. It is located across from the *Pennsylvania Station* between 32nd and 33rd Streets on the east side of Seventh Avenue.

From *Grand Central Station* take the subway "shuttle" (follow the green lights) to Times Square and then take either a downtown Express train or Local train to the Pennsylvania Station stop where there is an entrance directly into the Statler Hotel. The Governor Clinton, McAlpin, New Yorker and Martinique hotels are all in this region. Consult the map on the back of this program.

The *demonstrations* and *special exhibits* of the Society as well as the reading of one session of papers and the *special movie program* will be held on Thursday afternoon in Fayerweather Hall and Schermerhorn Hall of Columbia University.

This may be reached by taking the 7th Avenue uptown subway train to 116th Street. Take the Van Cortlandt Park Express. *Do not take the Bronx Park train.*

The Biologists' Smoker will take place Thursday evening at the American Museum of Natural History and can be reached by taking the 8th Avenue subway train uptown to 78th Street — Local train; or Express to 59th Street and then change to the Local. The Subway station opens directly into the downstairs Museum Foyer.

DINNER TICKETS

Tickets for the Zoologists' dinner on Wednesday evening will be on sale at the A.A.A.S. registration desk in the Hotel Statler and also outside the grand ballroom on Wednesday morning, December 28th. Since the hotel insists on a guaranteed number of persons attending the dinner, it is urged that all persons planning to attend the dinner purchase tickets before 12 o'clock noon on Wednesday.

This dinner will take place at the Hotel Martinique, Broadway and 32nd Street, at 6:00 P.M. on Wednesday evening.

DISCUSSION OF PAPERS

Due to the very large number of papers submitted for reading at this meeting of our Society, more papers have been listed for the various sessions than can be read and still allow time for discussion. Therefore, all those who have requested the full 15 minutes for the reading of their papers are urged to limit the time of presentation to 12 minutes if they wish to provide time for discussion.

COMMITTEE ON AID TO FOREIGN SCIENTISTS

Aid to foreign scientists has been officially undertaken by several biological societies, notably the American Physiological Society and the Genetics Society of America. The American Society of Zoologists has materially helped the Zoological Station at Naples by collecting individual subscriptions, but has undertaken no general program of aid. Recently the

American Institute of Biological Sciences has brought up the question of a more general aid program to take the form of financial help to needy individuals, as well as the collection of scientific reprints and journals to be sent to certain centers.

President Chambers has appointed a committee under the chairmanship of Dr. H. H. Plough, Department of Biology, Amherst College, Amherst, Massachusetts, to study the matter and make recommendations at the business meeting of the Society of Zoologists in New York. Those who have suggestions concerning aid to foreign zoologists may communicate with Dr. Plough by letter at any time, or during this session of the Society.

P R O G R A M

WEDNESDAY, DECEMBER 28th

- 9:15 A.M. Joint session with the Genetics Society of America.
Symposium on "*Experimental Cell Research*," Dr. Robert Chambers, presiding. Program on page 8. Grand Ballroom, Statler Hotel.
- 12:00 M. *Annual Business Meeting of Section F, A.A.A.S.*, Grand Ballroom, Statler Hotel.
- 12:10 P.M. *Annual Business Meeting of the American Society of Zoologists.*
- 2:00 P.M. Section A. *General Physiology*. Papers 5 to 16. Parlor 1, Statler Hotel.
Section B. *Embryology*. Papers 17 to 27. Parlor 2, Statler Hotel.
Section C. *Cytology*. Papers 28 to 40. Parlor A, Statler Hotel.
Section D. *Protozoology and Parasitology*. Papers 41 to 53. Parlor B, Statler Hotel.
- 6:00 P.M. Zoologists' Dinner, Gold and Oak Rooms, Hotel Martinique.

THURSDAY, DECEMBER 29th

- 9:00 A.M. Section A. Joint session with the Ecological Society of America. A session of papers (54 to 67) on "*Animal Behavior and Sociobiology*," Georgian Room, Statler Hotel.
- Section B. *General Physiology*. Papers 68 to 79. Parlor 1, Statler Hotel.
- Section C. *Endocrinology*. Papers 80 to 90. Parlor A, Statler Hotel.
- Section D. *Cellular Physiology*. Papers 91 to 102. Parlor B, Statler Hotel.

THURSDAY, DECEMBER 29TH (continued)

2:00 P.M. Section A. *General Morphology, Ecology, Evolution and Genetics.* Papers 103 to 114. Room 902, Schermerhorn Hall, Columbia University.

Section B. Joint session with the Genetics Society of America. *General Demonstrations.* Zoologists' demonstrations 115 to 151, Rooms 652 and 654. Geneticists' demonstrations 1 to 20, Rooms 903 and 913, both in Schermerhorn Hall, Columbia University.

Section C. *Demonstrations by Motion Pictures.* Papers 152 to 160. Room 201, Fayerweather Hall, Columbia University.

9:00 P.M. *Biologists' Smoker.* Sponsored by the American Society of Naturalists, American Museum of Natural History.

SPECIAL EVENTS*For Animal Behaviorists and Sociobiologists*

12:30 P.M. *Luncheon and Business Meeting* of the Committee for the Study of Animal Societies under Natural Conditions. Statler Hotel.

3:30 P.M. *Open House and Tea* for those interested in Animal Behavior Studies. Department of Animal Behavior, American Museum of Natural History, followed by:

4:30 P.M. *Informal Symposium.* Problems of Interpretation of Animal Behavior Studies. T. C. Schneirla, Chairman.

FRIDAY, DECEMBER 30th

9:00 A.M. Section A. *General Physiology.* Papers 161 to 174. Parlor 1, Statler Hotel.

Section B. *Endocrinology.* Papers 175 to 186. Parlor 2, Statler Hotel.

Section C. *Embryology.* Papers 187 to 199. Parlor A, Statler Hotel.

Section D. *Cellular and General Physiology.* Papers 200 to 212. Parlor B, Statler Hotel.

2:00 P.M. Symposium on "*Steroid Hormones and Sex Differentiation in Vertebrates.*" Sponsored by A.A.A.S. Dr. R. K. Meyer, presiding. Program on page 24. Grand Ballroom, Statler Hotel.

LIST OF TITLES

WEDNESDAY MORNING SESSION, DECEMBER 28TH

9:15 A.M. Grand Ballroom, Statler Hotel

Joint session with Genetic Society of America

Symposium

"Experimental Cell Research"

DR. ROBERT CHAMBERS, presiding

1. The physical nature of the living cell with special reference to the fertilized echinoderm ovum. Robert Chambers, Washington Square College.
2. Cellular aspects of embryonic differentiation. Donald P. Costello, University of North Carolina.
3. The problem of specificity in cytochemical reactions. Stanley H. Bennett, University of Washington.
4. Cell physiology — present and future. M. J. Kopac, Washington Square College.

WEDNESDAY NOON SESSION, DECEMBER 28TH

Business Meeting

The Annual Business Meeting of Section F, A.A.A.S. will be held at 12:00 noon in the Grand Ballroom of the Statler Hotel immediately following the Symposium. The Annual Business Meeting of the American Society of Zoologists will be held in the same room at 12:10 P.M. immediately following the meeting of Section F.

WEDNESDAY AFTERNOON SESSION, DECEMBER 28TH

This session will be held in 4 sections, as shown below.

Section A. *General Physiology*, 2:00 P.M.

Parlor 1, Statler Hotel

L. V. HEILBRUNN, presiding

5. The metabolism of starved nymphs of the grasshopper, *Chortophaga viridifasciata*. Daniel Ludwig, New York University, University Heights. (Lantern; 15 min.)

6. The effect of temperature on the growth and oxygen consumption of the chick embryo. Donald Greiff and Henry Pinkerton, St. Louis University. (Lantern; 15 min.)
7. A temperature differentiation of the dual action of amyl carbamate on frog nerve. Frederick Crescitelli with P. B. Hollander and Alice J. Olmstead, University of California, Los Angeles. (Lantern; 15 min.)
8. On the spawning behavior of oysters and of *Venus mercenaria* with special reference to the effects of spermatie hormones. Thurlow C. Nelson and H. H. Haskin, Rutgers University. (Lantern; 15 min.)
9. Some reactions of the tracheal system of *Phormia* larvae. John B. Buck and Margaret L. Keister, National Institutes of Health. (Lantern; 15 min.)
10. Reversibility of tracheal filling in *Sciara* larvae. Margaret L. Keister and John B. Buck, National Institutes of Health. (Lantern; 15 min.)
11. Extreme longevity in Rotifers. Albert I. Lansing, Washington University. (Lantern; 15 min.)
12. Isolation and properties of rat liver nuclei. Karl M. Wilbur, Norman G. Anderson and Max V. Skeen, Duke University. (Lantern; 10 min.)
13. The relationship between carbonic anhydrase and growth in mollusks. Norman G. Anderson and Karl M. Wilbur, Duke University. (Lantern; 5 min.)
14. Assay for the growth and differentiation hormone of Lepidoptera by the method of tissue culture. Edmund L. Schmidt and Carroll M. Williams, Harvard University. (Introduced by Henry G. Bigelow.) (Lantern; 15 min.)
15. Extracellular fluid volume of the rat. Roy V. Talmage, R. C. Frost, R. J. Shalek, J. H. Burr and F. A. Garrett, Rice Institute. (Lantern; 15 min.)
16. Antibiotic effects of fowl leucocytes in vitro. C. G. Grand, Washington Square College. (Introduced by Robert Chambers.) (Lantern; 10 min.)

WEDNESDAY AFTERNOON SESSION, DECEMBER 28TH

Section B. *Embryology*, 2:00 P.M.

Parlor 2, Statler Hotel

B. H. WILLIER, presiding

17. Inhibition of melanophore migration in the white axolotl. H. Clark Dalton, The Carnegie Institution of Washington. (Lantern; 15 min.)
18. Innervation of fin transplants in *Opsanus tau*. T. H. Waterman and J. S. Nicholas, Yale University. (Lantern; 15 min.)
19. Development of respiratory enzymes in rat muscle. S. C. Shen, Yale University. (Introduced by A. Petrunkevitch.) (Lantern; 15 min.)
20. Experimental modification of cholinesterase development in the midbrain of *Amblystoma punctatum*. E. J. Boell and S. C. Shen, Yale University. (Lantern; 15 min.)
21. Behavior of components of the early embryo of the mouse in culture and in the anterior chamber of the eye. Clifford Grobstein, National Cancer Institute. (Lantern; 15 min.)

22. The effects of various abnormal agents applied to the early developmental stages of *Hemichromis bimaculata* and their theoretical significance. Robert S. McEwen, Janet B. Briggs and Margaret S. Gilbert, Oberlin College. (Lantern; 15 min.)
23. Studies of grafted heads of *Rana pipiens*. Jerry J. Kollros, State University of Iowa. (Lantern; 15 min.)
24. Evidence for structural specificity in basic proteins. Charles B. Metz, Mary T. Foley and Joanne E. Donovan, Yale University. (Lantern; 15 min.)
25. An analysis of the effects of certain anti-organ sera on the development of the early chick blastoderm in vitro. James D. Ebert, The Johns Hopkins University. (Introduced by B. H. Willier.) (Lantern; 15 min.)
26. Determination of structural patterns in the spinal cord of the chick embryo studied by transplantations between brachial and adjacent levels. Byron S. Wenger, Washington University. (Introduced by Viktor Hamburger.) (Lantern; 15 min.)
27. X-ray induced developmental abnormalities in the mouse and their use in the analysis of embryological patterns. Liane Brauch Russell, Oak Ridge National Laboratory. (Introduced by W. L. Russell.) (Lantern; 15 min.)

WEDNESDAY AFTERNOON SESSION, DECEMBER 28TH

Section C. *Cytology*, 2:00 P.M.

Parlor A, Statler Hotel

C. W. METZ AND FRANZ SCHRADER, presiding

28. An evaluation of autoradiographs of bull sperm. David W. Bishop, University of Massachusetts. (Lantern; 10 min.)
29. Studies on the histochemical localization of cholinesterase in nerve tissue of the dog. Walter L. Hard and Alvin C. Peterson, University of South Dakota. (Lantern; 12 min.)
30. An experimental study of somatic reduction in the mosquito. Joseph E. Schuh, Fordham University. (Introduced by C. A. Berger.) (Lantern; 7½ min.)
31. A study of the lampbrush chromosomes of some salamanders. M. A. Fontanella, Fordham University. (Introduced by C. A. Berger.) (Lantern; 7½ min.)
32. Chromosome structure as revealed by tryptic digestion. B. P. Kaufmann, The Carnegie Institution of Washington, and R. C. MacDuffee, Army Medical Department Research and Graduate School. (Lantern; 15 min.)
33. The effect of visible light on the survival of ultra-violet treated microconidia of *Neurospora crassa*. Sol H. Goodgal, The Johns Hopkins University. (Introduced by Mary E. Rawles.) (Lantern; 15 min.)
34. The effects of carbon dioxide and nitrogen gas on mitosis as observed in living neuroblasts of grasshopper embryo. Mary E. Gaulden, J. Gordon Carlson and Samuel R. Tipton, Oak Ridge National Laboratory and University of Tennessee. (Introduced by Maurice Whittinghill.) (Lantern; 15 min.)

35. Electron microscope studies on the structure of cardiac muscle. H. W. Beams, T. C. Evans, C. D. Janney and W. W. Baker, State University of Iowa. (Lantern; 10 min.)
36. The desoxypentose nucleic acid content of animal nuclei. Hewson H. Swift, Columbia University. (Introduced by A. W. Pollister.) (Lantern; 15 min.)
37. Some factors influencing tumor incidence in *Drosophila melanogaster*. Ernest W. Hartung, Rhode Island State College. (Lantern; 15 min.)
38. Human fetal brain cells, 8 days after death, grown in tissue cultures. Mary Jane Hogue, University of Pennsylvania. (Lantern; 10 min.)
39. Cytological changes in the livers of obese mice subjected to a prolonged fast. J. Walter Wilson and Elizabeth H. Ledue, Brown University. (Lantern; 15 min.)
40. Some conclusions reached from microdissection studies of the dividing neuroblast of the grasshopper embryo. J. Gordon Carlson, Oak Ridge National Laboratory and University of Tennessee. (Lantern; 15 min.)

WEDNESDAY AFTERNOON SESSION, DECEMBER 28TH

Section D. *Protozoology and Parasitology*, 2:00 P.M.

Parlor B, Statler Hotel

WILLIS H. JOHNSON AND HORACE W. STUNKHARD, presiding

41. An abnormal type of development in the life cycle of *Tokophrya infusionum*. Maria A. Rudzinska, Washington Square College. (Introduced by Robert Chambers.) (Lantern; 10 min.)
42. Survival of *Astasia longa* following X-irradiation. Henry W. Schoenborn, University of Georgia and Oak Ridge National Laboratory. (Lantern; 10 min.)
43. Effect of visible light on growth of *Tetrahymena geleii*. Austin Phelps, University of Texas. (Lantern; 10 min.)
44. The role of thiamine in the metabolism of *Tetrahymena geleii*. Irving A. Tittler, Brooklyn College. (15 min.)
45. Temperature and population longevity in *Tetrahymena geleii*. E. G. Stanley Baker and Sister Jeanne d'Arc Schleichter, The Catholic University of America. (Lantern; 10 min.)
46. Effect of allyl isothiocyanate upon oxygen consumption and carbon dioxide output of *Spirostomum ambiguum*. Harold E. Finley and Kenneth A. Tracey, Howard University. (Lantern; 5 min.)
47. Encystment studies on *Spirostomum ambiguum*. Harold E. Finley and Henry A. Wise, Howard University. (Lantern; 5 min.)
48. An analysis of the effects of a sacculinid on the external morphology of *Callinectes sapidus Rathbun*. Edward G. Reinhard, The Catholic University of America. (Lantern; 15 min.)
49. The ability of the avian malaria parasite, *Plasmodium lophurae*, to infect erythrocytes of distantly related species. R. Barclay McGhee, Rockefeller Institute. (Introduced by N. R. Stoll.) (Lantern; 10 min.)

50. Fungi associated with tumor tissues. Irene Cory Diller, Institute of Cancer Research, Philadelphia. (Lantern; 15 min.)
51. Studies on the extracellular development *in vitro* of an intracellular parasite (avian malaria). William Trager, Rockefeller Institute. (Lantern; 10 min.)
52. Frequent but apparently harmless occurrence of sarcosporidiosis in healthy beef. Hsi Wang, American Meat Institute Foundation, University of Chicago. (Lantern; 15 min.)
53. Crustacean parasites from Bimini and the Carolina Coast. A. S. Pearse, Duke University. (Lantern; 15 min.)

THURSDAY FORENOON SESSION, DECEMBER 29TH

This session will be held in 4 sections, as shown below.

Section A. *Animal Biology and Sociobiology*, 9:00 A.M.

Joint session with the Ecological Society of America.

Georgian Room, Statler Hotel

DR. J. P. SCOTT, presiding

54. Certain aspects of the development and behavior of the Wyoming moose — *Alces americana shirasi*. R. H. Denniston, University of Wyoming. (Motion picture; 15 min.)
55. Patterns of cooperative behavior in a herd of 14 giant tortoises at the Bronx Zoo. L. T. Evans and J. Quaranta, New York Zoological Society. (Motion picture; 8 min.)
56. The role of the distal tip of the gonopodium during the copulatory act of the viviparous teleost, *Platypoecilus maculatus*. Eugenie Clark, Lester R. Aronson and Myron Gordon, American Museum of Natural History and New York Zoological Society. (Lantern; 15 min.)
57. The influence of social domination on receptivity in the domestic fowl. A. M. Guhl, Kansas State College. (Lantern; 15 min.)
58. Hunting and herding behavior in dogs. Benson E. Ginsburg and Mildred J. Zamis, The University of Chicago and Jackson Memorial Laboratory. (10 min.)
59. The formation of conditioned avoidance responses in young puppies. John L. Fuller, Clarice Easler and Edwin Banks, Jackson Memorial Laboratory. (Lantern; 15 min.)
60. Influence of space and time on the social behavior of the rat. John B. Calhoun, Johns Hopkins University. (15 min.)
61. Radioisotopes as tools for the study of animals under natural conditions. Donald R. Griffin, Cornell University. (Lantern; 8 min.)
62. Bird activity in the continuous daylight of arctic summer. Martin Karplus, Harvard University. (Introduced by D. R. Griffin.) (Lantern; 7 min.)

63. Response latency and habit strength in relationship to spontaneous fighting in C57 black mice. Emil Frederieson, Jackson Memorial Laboratory. (Introduced by J. P. Scott.) (Lantern; 7 min.)
64. Analysis of individual differences in patterns of conflict behavior in dogs. J. P. Scott and Margaret Charles, Jackson Memorial Laboratory. (8 min.)
65. A discrimination study with the African elephant. Joseph A. Murnin and Dieter Bueckhardt, New York Zoological Society. (Introduced by T. C. Schneirla.) (Motion picture; 15 min.)
66. The color discrimination of *Testudo vicina*. John V. Quaranta, New York Zoological Society. (Introduced by C. C. Little.) (Motion picture; 8 min.)
67. A study of dominance order in a herd of captive giant tortoises (Testudinidae) resident at the Bronx Zoo. John V. Quaranta and L. T. Evans, New York Zoological Society. (Introduced by C. C. Little.) (Motion picture; 7 min.)

THURSDAY FORENOON SESSION, DECEMBER 29TH

Section B. *General Physiology*, 9:00 A.M.

Parlor 1, Statler Hotel

E. J. BOELL, presiding

68. Some effects of antibiotics on *Daphnia*. Bertil G. Anderson, West Virginia University. (Lantern; 15 min.)
69. Further studies on growth factors for carnivorous ciliates. Daniel M. Lilly, Anthony Pignataro and Kenneth Heilshorn, St. John's University. (Lantern; 15 min.)
70. Unusual properties of the succinoxidase system in the cecropia silkworm. Richard C. Sanborn and Carroll M. Williams, Harvard University. (Lantern; 15 min.)
71. Amino acid and nucleic acid relationships in *Drosophila*. Taylor Hinton, John Ellis and D. L. Theriault, Amherst College. (Lantern; 15 min.)
72. Electrical control of axial polarity in a regenerating annelid. Gordon Marsh and H. W. Beams, State University of Iowa. (Lantern; 15 min.)
73. Quantitative antibody studies in immature and adult chickens. Stata Norton and Harold R. Wolfe, University of Wisconsin. (Lantern; 12 min.)
74. The effect of splenectomy on precipitin formation in chickens. Harold R. Wolfe, Stata Norton, Eleanor Springer, Morris Goodman and C. A. Herrick, University of Wisconsin. (Lantern; 10 min.)
75. The effect of adrenalectomy on the oxygen consumption of testis of testosterone-treated normal rats. Jorge Gonzalez Q. and Clifford A. Angerer, Ohio State University. (Lantern; 12 min.)
76. Phototropic responses in *Ameiurus* embryos and the associated retinal morphology. Philip B. Armstrong, Syracuse University and Marine Biological Laboratory. (Lantern; 12 min.)

77. Effect of acid-base changes on visual thresholds. Charles D. Hendley, Ohio State University. (Introduced by I. Rothschild.) (Lantern; 10 min.)
78. Fat metabolism in the arctic ground squirrel. Charles G. Wilber, St. Louis University. (Lantern; 15 min.)
79. Biochemical changes during the embryonic development of *Popillia japonica*. Fred Rothstein, New York University. (Introduced by H. W. Stunkard.) (Lantern; 15 min.)

THURSDAY FORENOON SESSION, DECEMBER 29TH

Section C. *Endocrinology*, 9:00 A.M.

Parlor A, Statler Hotel

CARL R. MOORE, presiding

80. Relative sensitivity of the immature and pregnant mouse for assay of unfractionated pituitary glands. A. J. Ladman, Jackson Memorial Laboratory. (Introduced by M. N. Runner.) (Lantern; 15 min.)
81. Estrogen levels in the non-ovulating rabbit. Clara Eddy Hamilton, University of Georgia. (Lantern; 10 min.)
82. The effect of dietary deficiencies and endocrine alterations on the apyrase and the d-amino acid oxidase of rat tissues. Samuel R. Tipton, Frances M. Colvin and Kurt Weiss, University of Tennessee. (Lantern; 10 min.)
83. Spectrophotometric analysis of feather pigments from adult brown Leghorn capons and thyroidectomized roosters. Ben B. Blivaiss and Sherwin Zalman, Chicago Medical School. (Lantern; 15 min.)
84. Effect of adrenocorticotrophin on the adrenal gland of thiouracil treated rats. M. X. Zarrow and I. G. Zarrow, Purdue University. (Lantern; 10 min.)
85. Effect of ultrasonic vibrations on the molting response of *Triturus viridescens*. Hugh Clark, University of Connecticut. (Lantern; 10 min.)
86. Some factors influencing the cholesterol content of the interstitial tissue of the immature rat ovary. Edward G. Rennels, Harvard University. (Introduced by A. B. Dawson.) (Lantern; 15 min.)
87. The androgenic activity of ovarian transplants to the seminal vesicle of the castrated adult male rat. Seymour Katsh, Washington Square College. (Introduced by H. A. Charipper.) (Lantern; 15 min.)
88. Changes in reproductive and metabolic functions in mice given radiotoxic dosages of I^{131} . Aubrey Gorbman and Hilda Beim, Barnard College. (Lantern; 15 min.)
89. The relation of the numbers of corpora lutea and of embryos in wild rats. Octavia Hall and David E. Davis, University of Texas and Johns Hopkins University. (Lantern; 15 min.)
90. The relation of day length and gonadal regression to postnuptial molt in the white-throated sparrow and other frangillids. Albert Wolfson, Northwestern University. (Lantern; 15 min.)

THURSDAY FORENOON SESSION, DECEMBER 29TH

Section D. *Cellular Physiology*, 9:00 A.M.

Parlor B, Statler Hotel

A. K. PARFART, presiding

91. Utilization of atmospheric nitrogen by the animal microorganism, *Colpidium campylum*. Gerald R. Seaman, Creighton Medical School. (Introduced by John Frisch.) (Lantern; 15 min.)
92. Fourteen-day rhythms and surface relationships of leucocytes and amebas. A. A. Schaeffer and Carol Honegger, Temple University. (Lantern; 15 min.)
93. Histories of implants of foreign substances in frog tadpoles. Carl Caskey Speidel, University of Virginia. (Lantern; 15 min.)
94. Effect of ultraviolet radiation on the rate of cell division of *Arbacia* eggs and the enhancement of recovery by visible light. Harold F. Blum, J. P. Price, J. C. Robinson and G. M. Loos, National Cancer Institute. (Lantern; 15 min.)
95. *Arbacia* sensitivity to dimethylated dioxypurines. Ralph Holt Cheney, Brooklyn College. (Lantern; 15 min.)
96. Gradual inhibition of a population of dividing sea-urchin eggs by hypoxanthine and theophylline. Ivor Cornman, George Washington University. (Introduced by A. F. Shull.) (7 min.)
97. Concerning the significance of the affinity of motor end-plates for janus green B. John H. Welsh and Sumner Zacks, Harvard University. (Lantern; 15 min.)
98. The effects of methylene blue plus urethane (ethyl carbamate) upon the oxygen uptake of embryonic cells. Joseph H. Bodine, State University of Iowa. (Lantern; 15 min.)
99. An experimental study of mitotic gelation. L. V. Heilbrunn, W. L. Wilson and Drusilla Harding, University of Pennsylvania. (Lantern; 15 min.)
100. Some effects of urethane (ethyl carbamate) on mitosis in the trout blastoderm. Helen I. Battle and Margaret A. Laing, University of Western Ontario. (Lantern; 15 min.)
101. Growth factor requirements of *Tetrahymena geleii* E. Alfred M. Elliott, University of Michigan. (Lantern; 10 min.)
102. Comparative amino acid metabolism of *Tetrahymena geleii* E. Alfred M. Elliott and J. F. Hogg, University of Michigan. (Lantern; 5 min.)

THURSDAY AFTERNOON SESSION, DECEMBER 29TH

This session will be held in three sections as shown below.

Section A. *General Morphology, Ecology, Evolution and Genetics*, 2:00 P.M.

Room 902, Schermerhorn Hall, Columbia University

W. H. COLE AND B. R. COONFIELD, presiding

103. Notes on the rare Archiannelid genus *Dinophilus* in the United States. E. Ruffin Jones, Jr., University of Florida and Frederick F. Ferguson, University of Washington. (10 min.)

104. Motor nerve endings in muscle of the cockroach. Elizabeth A. Weiant, Tufts College. (Introduced by K. D. Roeder.) (Lantern; 15 min.)
105. Respiratory area of the toadfish. I. E. Gray, Duke University. (Lantern; 15 min.)
106. The morphology of the lymphatics of the rabbit ovary and their possible relation to ovulation. J. H. Burr, Jr, and Joseph I. Davies, Rice Institute. (Introduced by Theo. L. Jahn.) (8 min.)
107. The affinities of the Bryozoa. F. H. Pike, Columbia University. (10 min.)
108. The relative position of the Cetacea among the orders of Mammalia as indicated by precipitin tests. Alan Boyden and Douglas Gemeroy, Rutgers University. (Lantern; 15 min.)
109. Studies in the physiology of commensalism. I. The Polynoid genus *Arctonoe*. Demorest Davenport, University of California, Santa Barbara College. (15 min.)
110. Differences in toleration of drying between species of termites (Reticulitermes). Margaret J. Strickland, University of Chicago. (Introduced by H. E. Finley.) (5 min.)
111. A case of cytoplasmic transmission in *Daphnia pulex* DeGeer. A. S. Bradshaw, Cornell University. (Introduced by D. C. Chandler.) (10 min.)
112. Improved axenic cultivation of *Rhabditis briggsae* Dougherty and Nigon, 1949 (Nematoda). Ellsworth C. Dougherty, University of California (Berkeley) and California Institute of Technology, James C. Raphael, Jr. and Carlota H. Alton, University of California (Berkeley). (Lantern; 7 min.)
113. Attempts at hybridizing two closely related hermaphroditic species of the nematode genus *Rhabditis*. Victor Nigon, Faculté des Sciences P. C. B., Paris, and Ellsworth C. Dougherty, University of California (Berkeley) and California Institute of Technology. (Lantern; 8 min.)
114. Intra-uterine death time in semi-sterile mice. Eileen M. Otis, University of Rochester. (Introduced by Donald R. Charles.) (Lantern; 15 min.)

THURSDAY AFTERNOON SESSION, DECEMBER 29TH

Section B. *Demonstrations by Zoologists*, 2:00 P.M.

Joint session with the Genetics Society of America.

Rooms 652 and 654, Schermerhorn Hall, Columbia University

DR. AUBREY GOREMAN, in charge

115. X-ray induced greying in the mouse with reference to melanoblasts. Herman B. Chase and Virginia W. Smith, Brown University.
116. Hair growth and pigmentation in relation to biotin deficiency. Harold Rauch, Brown University. (Introduced by Elizabeth B. Chase).
117. Visualization of dehydrogenase activity in tissues. Maurice M. Black, M.D. New York Medical College. (Introduced by Secretary.)

118. Structural make-up of terminal vascular bed. Benjamin W. Zweifach, Cornell University Medical College.
119. The effect of injection dosage level of lyophilized mouse tissue on the subsequent growth of a tumor homoiotransplant. Nathan Kaliss and Olive Newton, Jackson Memorial Laboratory.
120. A study of antibody production in inbred strains of mice in connection with the growth of transplanted tumors. George E. Jay, Jr., and Nathan Kaliss, Jackson Memorial Laboratory.
121. Staining of nucleic acids by Azure A. Martin Flax and Arthur W. Pollister, Columbia University.
122. Photometric analysis of cells. Arthur W. Pollister, Montrose J. Moses and Cecilie Leuchtenberger, Columbia University.
123. A histo-photoelectric method of collagen determination in muscles. Hsi Wang, American Meat Institute Foundation, University of Chicago.
124. Demonstration of tissue sections prepared by rapid freezing-sectioning technique. A. B. Taylor and F. B. Adamstone, University of Illinois.
125. The effect of added dietary tryptophane on the occurrence of diethylstilbestrol induced mammary cancer in the rat. W. F. Dunning, M. R. Curtis and M. E. Maun, Detroit Institute of Cancer Research.
126. The immediate source of the primary interstitial tissue of the ovary of the infantile rat. Alden B. Dawson, Harvard University.
127. Demonstrating amphibian cleavages with dry mounts. Mychyle W. Johnson, Duke University.
128. A study of pattern formation in a Bermuda coral reef fish. H. B. Goodrich, Ruth Hine and Jack Reynolds, Wesleyan University.
129. New mutations and linkage studies in the house mouse (*Mus musculus*). M. M. Dickie, D. E. Kelton, J. H. Fielder, A. M. Ingalls and G. D. Snell, Jackson Memorial Laboratory.
130. Cyclic changes in the epithelium of the vagina, cervix and uterine body of the golden hamster correlated with vaginal smears and the effects thereon of castration and estrone administration. George C. Kent, Jr. and J. Harvey Roberts, Louisiana State University.
131. Maturity indicators in the knee of the rabbit. Dorcas D. Crary, Jackson Memorial Laboratory. (Introduced by P. B. Sawin.)
132. A study of the central nervous system of flightless *Drosophila melanogaster*. Maxwell E. Power, Kenyon College.
133. Methods of culturing canivorous protozoa under bacteria-free conditions. Daniel M. Lilly, St. John's University.
134. On binary fission in *Spirostonum ambiguum*. Harold E. Finley and Avery W. Beverly, Howard University.
135. An extra postzygotic nuclear division in *Paramecium caudatum*. William F. Diller, University of Pennsylvania.
136. The effect of ultraviolet light upon the structure of the macronucleus of *Paramecium aurelia*. R. F. Kimball, Oak Ridge National Laboratory.
137. Extrusion of nuclear material in irradiated amphibian oocytes. William R. Duryee, National Cancer Institute.
138. Transfer of I^{131} to the nursing mouse. Roberts Rugh, Columbia University.

139. Living circulation in the mesentry of a rat. B. W. Zweifach, The New York Hospital.
140. Vital staining. Leonor Michaelis (deceased), Member Emeritus, Rockefeller Institute.
141. The morphological basis for the diagnosis of cancer by exfoliative cytology. George H. Papanicolaou, Cornell University Medical College. (Introduced by Secretary.)
142. The structural and functional relationships between the red and green pigments of protoplasm. S. Granick, Rockefeller Institute. (Introduced by Secretary.)
143. The nature of the Golgi apparatus. George E. Palade, Rockefeller Institute. (Introduced by Robert Chambers.)
144. The formation of certain connective tissue fibers. Parker Vanamee and Keith R. Porter, Rockefeller Institute.
145. Identification of "neuro-carcinoma" cells in the human cervix using a surface-biopsy modification of the Papanicolaou technique. J. Ernest Ayre, McGill University. (Introduced by Secretary.)
146. Contrast control in phase microscopy from appropriate choice of mounting medium. Oscar W. Richards, American Optical Company.
147. Tridimensional cellular structure. Oscar W. Richards, American Optical Company.
148. The problem of chemoautotrophy in *Chilomonas paramecium*. W. B. Cosgrove, New York University and the State University of Iowa. (Introduced by R. P. Hall.)
149. Synthesis of members of the vitamin-B complex by *Chilomonas paramecium*. George G. Holz, Jr., New York University and University of California at Los Angeles. (Introduced by R. P. Hall.)
150. Comparative morphology of certain ciliates in the Colpidium-Glaucoma-Tetrahymena group. John O. Corliss, New York University. (Introduced by R. P. Hall.)
151. Studies on the biotic potential of *Drosophila*. John M. Carpenter, University of Tennessee.

Demonstrations by Geneticists, 2:00 P.M.

Rooms 903-913, Schermerhorn Hall, Columbia University.

- Somatic eliminations of ring chromosomes in *Drosophila melanogaster*. Braver, Gerald and Jerry L. Blount, University of Missouri, Columbia, Mo.
- Differentiated chromosomes in several genera of the Solanaceae (preliminary observations.) Brown, Spencer W., University of California, Berkeley, Calif.
- Inheritance and selection of insulin-resistance in mice. Chase, Herman B., Brown University, Providence, R. I.
- The genetics of histogenic type variegation in plants. Dermen, Haig, Bureau of Plant Industry, Beltsville, Md.
- The progeny of triploid axolotls. Fankhauser, G. and R. R. Humphrey, Princeton University, Princeton, N. J. and University of Buffalo, Buffalo, N. Y.

- The genetic subdivision of the lozenge locus in *Drosophila melanogaster*. Green, M. M. and K. C. Green, University of Missouri, Columbia, Mo.
- A new chromomeric map of the X chromosome in *Drosophila melanogaster*. Griffen, A. B. and Mary Warters, University of Missouri, Columbia, Mo.
- The position effects Bar, white-mottled, and eubitus interruptus in *Drosophila melanogaster*. Griffen, A. B., Mary Warters and James D. Mohler, University of Missouri, Columbia, Mo. and Centenary College, Shreveport, La.
- The effect of some folic acid analogues on mitosis. Himes, Marion M., Columbia University, New York, N. Y.
- Cytological studies in the virilis group of *Drosophila* I-Inversion variations in the americana-texana-novamexicana complex. Hsu, T. C., University of Texas, Austin, Tex.
- Analysis of cell structure by digestion with enzymes. Kaufmann, Berwind P., Helen Gay and Margaret McDonald, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.
- On the genetical activity of the Y chromosome in *Drosophila buseki*. Krivshenko, Jakov, University of Missouri, Columbia, Mo.
- Disruption of mitosis in onion root cells by thyroid powder. Mittler, Sidney and Robert H. Herman, Illinois Institute of Technology, Chicago, Ill.
- Chemical differentiation of the larval mid gut of *Drosophila*. Poulson, D. F., Yale University, New Haven, Conn.
- Imitation of specific pattern characters of *Hippodamia quinquesignata* through residual variation in *H. convergens*. Shull, A. Franklin, University of Michigan, Ann Arbor, Mich.
- Non-random distribution of chromosome fragmentation in unirradiated *Trillium*. Sparrow, A. H. and E. Christensen, Brookhaven National Laboratory, Upton, L. I., N. Y.
- Demonstrations of human pedigrees. Stephens, F. E., F. H. Tyler, D. A. Dolowitz and Eldon J. Gardner, University of Utah School of Medicine, Salt Lake City, Utah.
- Transition of meiosis to mitosis and vice versa in cultures of excised antlers. Taylor, J. Herbert, University of Tennessee, Knoxville, Tenn.
- The telomeres of *Drosophila*. Warters, Mary and A. B. Griffen, Centenary College, Shreveport, La., and University of Missouri, Columbia, Mo.
- Mutant types in *Mormoniella*. Whiting, P. W., University of Pennsylvania, Philadelphia, Pa.

THURSDAY AFTERNOON SESSION, DECEMBER 29TH

Section C. *Demonstrations by Motion Pictures*, 2:00 P.M.

Room 201, Fayerweather Hall, Columbia University

DR. RALPH BUCHSBAUM, presiding

152. The effect of local x-ray irradiation upon development of various parts of the body of the young mouse. V. V. Brunst, Frank H. Figge and D. J. Barnett, University of Maryland Medical School. (14 min.)

153. Peripheral vascular reactions induced by x-irradiation of a portion of the wing of the bat, *Myotis lucifugus*. Douglas E. Smith, George Svihla and Harvey M. Patt, Argonne National Laboratory. (Introduced by T. N. Tahmisian.) (10 min.)
154. Artificial insemination of rabbits and transplantation of rabbit eggs. Min Cheuh Chang and G. Pincus, Worcester Foundation for Experimental Biology. (22 min.)
155. Nesting habits of the diamond-back terrapin. Irwin C. Kitchin, University of Georgia. (13 min.)
156. An auto-chromo-photomicrotome. Erling S. Hegre, Medical College of Virginia. (Introduced by S. S. Negus.) (15 min.)
157. Cine-photomicrographs of implants of tantalum in frog tadpoles. Carl C. Speidel, University of Virginia. (15 min.)
158. Reproductive behavior in the African mouthbreeding fish, *Tilapia macrocephala*. Lester R. Aronson and A. Marie Holz-Tucker, American Museum of Natural History. (30 min.)
159. The burying beetles. Lorus and Majorie Milne, University of New Hampshire. (8 min.)
160. Spermatozoa of the vertebrates. Edmond J. Farris, The Wistar Institute. (15 min.)

THURSDAY EVENING SESSION, DECEMBER 29TH

Biologists' Smoker, 9:00 P.M.

American Museum of Natural History

FRIDAY FORENOON SESSION, DECEMBER 30TH

This session will be held in 4 sections as shown below.

Section A. *General Physiology*, 9:00 A.M.

Parlor 1, Statler Hotel

J. H. BODINE, presiding

161. The effect of low environment atmospheric pressures on the cholesterol content of the blood in the pigeon. Louis A. Susca, New York Medical College. (Introduced by Secretary.) (10 min.)
162. Respiratory mechanism in the rat. F. H. McCutcheon, University of Pennsylvania. (Lantern; 15 min.)
163. Histopathology of x-rayed larval urodele tissues, exposed and maintained at different temperatures. John P. O'Brien, Marquette University. (Lantern; 8 min.)

164. Indirect effects of x-radiation on the wall of the developing mesencephalon of the chick embryo. M. J. Tobin and John P. O'Brien, Marquette University. (Lantern; 7 min.)
165. Further investigation of the influence of local x-ray irradiation on the tail development of the young axolotl, *Siredon mexicanum*. V. V. Brunst, E. A. Sheremetieva-Brunst, F. H. J. Figge and D. J. Barnett, University of Maryland. (Lantern; 14 min.)
166. The uptake of radiophosphorus by vaccinia virus and its associated components. Luolin S. Altenburg, The Rice Institute. (Introduced by H. J. Muller.) (15 min.)
167. The effects of x-radiation on the metabolic processes of the resting cell. Theodore N. Tahmisian, Argonne National Laboratory. (Lantern; 15 min.)
168. Effects of x-rays on I^{131} uptake in rats and mice. Titus C. Evans and Estelle S. Sobel, State University of Iowa. (Lantern; 10 min.)
169. Effects of x-rays on the mitotic stages in the epidermis of the newborn mouse. Estelle S. Sobel, State University of Iowa. (Introduced by Titus C. Evans.) (Lantern; 5 min.)
170. Reticulocytosis following injection of anoxic plasma in dogs. F. W. Kinard and D. W. Ellis. Medical College of South Carolina. (Lantern; 10 min.)
171. Independent occurrence of audiogenic seizures and middle-ear infections in inbred mice. Dorothea Starbuck Miller and Mildred J. Zamis, University of Chicago. (Lantern; 10 min.)
172. A suggested mechanism of convulsive seizures, derived from observations upon peripheral nerve. J. E. P. Toman, M. D. Greenhalgh and L. M. Henrie, University of Utah College of Medicine. (Lantern; 15 min.)
173. The electrical effects of tolserol (myanesin). R. Beutner and T. C. Barnes, Hahnemann Medical College and Hospital of Philadelphia. (Lantern; 8 min.)
174. Animal experiments supporting the electrical theory of drug action. T. C. Barnes and R. Beutner, Hahnemann Medical College. (Lantern; 15 min.)

FRIDAY FORENOON SESSION, DECEMBER 30TH

Section B. *Endocrinology*, 9:00 A.M.

Parlor 2, Statler Hotel

F. L. HISAW, presiding

175. Development of the baculum or os penis in the long-tailed weasel. Philip L. Wright, Montana State University. (Lantern; 10 min.)
176. Changes in the thyroid gland of hamster embryos hypophysectomized by decapitation. Charles L. Foote and Florence M. Foote, Southern Illinois University. (Lantern; 15 min.)

177. Spontaneous rupture of the vaginal closure membrane during pregnancy in the guinea pig. William C. Young and Richard C. Webster, University of Kansas. (Lantern; 10 min.)
178. Effect of blood serum from hypophysectomized frogs, *Rana pipiens*, on ovulation in vitro. Paul A. Wright, University of Michigan. (15 min.)
179. Development of the dimorphic pigment spot of the Syrian hamster. Ruth E. Shrader, Yale University. (Introduced by Carroll A. Pfeiffer.) (Lantern; 15 min.)
180. Androgen production in the female brown Leghorn fowl. Elsie Taber, Medical College of South Carolina. (Lantern; 15 min.)
181. Resistance to cold and anoxia by young rats. H. A. Salhanick and J. L. Starr, Harvard University. (Introduced by Frederick L. Hisaw.) (Lantern; 15 min.)
182. Luteal function in the immature rat. C. T. G. King, M. Macauley, F. L. Hisaw and A. B. Dawson, Harvard University. (Introduced by Leigh Hoadley.) (Lantern; 15 min.)
183. The effect of physiological stress on water diuresis. J. H. Birnie and W. J. Eversole, Syracuse University. (Introduced by Robert Gaunt.) (Lantern; 15 min.)
184. Response of rat seminal vesicles to testosterone propionate suspension in methyl cellulose and testosterone adsorbed on aluminum phosphate. Harold Schraer and W. J. Eversole, Syracuse University. (Lantern; 10 min.)
185. Dissociation of metabolic and morphogenic thyroid functions in brown Leghorn fowl. Beatrice Mintz and L. V. Domm. (Lantern; 12 min.)
186. Differential mechanisms of pituitary activation by chemical agents. Charles H. Sawyer and J. E. Markee, Duke University. (Lantern; 15 min.)

FRIDAY FORENOON SESSION, DECEMBER 30TH

Section C. *Embryology*, 9:00 A.M.

Parlor A, Statler Hotel

PAUL WEISS, presiding

187. Hydrogen peroxide as a source of oxygen for asphyxiated newborn guinea pigs. James A. Miller, Faith S. Miller and Carolyn B. Farrar, Emory University. (Lantern; 15 min.)
188. Development of *Dasybranchus caducus* from egg to pre-adult. C. G. Bookhout, Duke University and Lerner Marine Laboratory. (Lantern; 15 min.)
189. Observations on cleavage and early germ layers in *Anolis carolinensis*. George W. D. Hamlett, Louisiana State University School of Medicine. (Lantern; 15 min.)
190. An analysis of the role of the apical ridge of ectoderm in the development of the limb bud in the chick. John W. Saunders, Jr., The Johns Hopkins University and University of Chicago. (Lantern; 15 min.)
191. Development in intestinal coiling in the anuran tadpole. Norman E. Kemp, University of Michigan. (Lantern; 15 min.)

192. Ovulation in the mouthbreeding cichlid fish, *Tilapia macrocephala* (Bleeker). Lester R. Aronson and A. Marie Holz-Tucker, American Museum of Natural History. (Lantern; 15 min.)
193. The reproductive potential of a single clone of *Pelmatohydra oligactis*. C. L. Turner, Northwestern University. (Lantern; 15 min.)
194. A comparison of the effects of sodium azide, thiourea, maleic acid, and malonic acid on developmental pattern in the sand dollar. Olin Rulon, Northwestern University. (Lantern; 15 min.)
195. Acid and alkaline phosphatase activity of the yolk and yolk sac of the developing chick embryo. Liselotte Mezger, Washington University. (Introduced by Florence Moog.) (10 min.)
196. The critical inhibitory voltage theory of growth limitation: further evidence. Gairdner Moment, Goucher College. (Lantern; 12 min.)
197. The use of placental and ovarian scars in determining reproductive history. H. T. Gier, Kansas State College. (10 min.)
198. Experimental study of artificial parthenogenesis in the frog. John R. Shaver, University of Missouri. (Introduced by M. J. Guthrie.) (15 min.)
199. Antigenic activity of cytoplasmic granules from embryonic tissue. John R. Shaver, University of Missouri. (Introduced by Daniel Mazia.) (10 min.)

FRIDAY FORENOON SESSSION, DECEMBER 30TH

Section D. *Cellular and General Physiology*, 9:00 A.M.

Parlor B, Statler Hotel

WILLIAM McELROY, presiding

200. The effect of environmental temperatures on cells. I. Effect upon the morphology and viability of neoplastic cells. Lawrence B. Walsh, Herman T. Blumenthal and Donald Greiff, St. Louis University. (Introduced by Walter C. Randall.) (15 min.)
201. The effect of prior injections of lyophilized normal mouse tissues on the subsequent growth of a transplanted tumor. Nathan Kaliss and G. D. Snell, Jackson Memorial Laboratory. (Lantern; 15 min.)
202. Synergistic and antagonistic influences on colchicine-poisoned sea-urchin eggs. Ivor Cornman, George Washington University. (Introduced by A. F. Shull.) (7 min.)
203. The production in mice of antibodies against mouse mitochondria and microsomes. G. D. Snell, Jackson Memorial Laboratory. (10 min.)
204. Interactions between nucleus and cytoplasm. William R. Duryee, National Cancer Institute, National Institutes of Health. (Lantern; 10 min.)
205. Adaptation of *Escherichia coli* to use of four-carbon dicarboxylic acids. Thomas C. Nelson, Columbia University. (Introduced by F. J. Ryan.) (15 min.)

206. *In vitro* transfer of radioiron across the nucleated and non-nucleated erythrocyte membrane. L. M. Sharpe, P. S. Krishnan and J. R. Klein. (Lantern; 12 min.)
207. Giant mitochondria in the flight muscles of insects. Mary Ishimoto and Carroll M. Williams, Harvard University. (Introduced by Alfred S. Romer.) (Lantern; 15 min.)
208. Glycogen content of various arctic animals. B. J. Sullivan, Boston College, and X. J. Musacchia, St. Louis University. (Introduced by Sr. Elizabeth Seton MacDonald.) (15 min.)
209. Surface areas of chromosomes in relation to their activity. Edward O. Dodson, University of Notre Dame. (Introduced by J. D. Mizelle.) (10 min.)
210. Effect of temperature on the lipids in the blood of *Chrysemys picta*. Joel R. Lieb and Charles G. Wilber. (Introduced by B. Luyet.) St. Louis University. (15 min.)
211. Effect of carbon dioxide on insect electroretinograms. Theodore L. Jahn and Lewis A. Kaplan, University of California at Los Angeles. (Lantern; 7 min.)
212. The blastocoel fluid in amphibian gastrulation. Louis T. Stableford, Lafayette College. (Introduced by B. W. Kunkel.) (Lantern; 10 min.)

FRIDAY AFTERNOON SESSION, DECEMBER 30TH

Grand Ballroom, Statler Hotel, 2:00 P.M.

Session arranged by A.A.A.S.

Symposium

"Steroid Hormones and Sex Differentiation in Vertebrates"

DR. R. K. MEYER, presiding

1. Hormone-induced sex inversion in fishes and amphibians. Emil Witschi, State University of Iowa. Discussants: Clifford Grobstein, National Cancer Institute and W. J. Eversole, Syracuse University.
2. Advances in our knowledge concerning the role of hormones in the sex differentiation of birds. L. V. Domm, The University of Chicago. Discussant: B. H. Willier, The Johns Hopkins University.
3. Embryonic sex hormones and differentiation of sex in mammals. C. R. Moore, The University of Chicago. Discussant: R. K. Burns, Jr., The Johns Hopkins University.

PAPERS PRESENTED BY TITLE

Animal Behavior

213. An experimental study of the possible sensory cues involved in the responses of *Alligator mississippiensis* to the sounds of the French horn. J. A. Murnin and J. V. Quaranta, New York Zoological Society. (Introduced by T. C. Schneirla.)

- 214. Notes on the social behavior of a herd of Nyalas, *Tragelaphus angasi* (Gray). Dieter Burekhardt, New York Zoological Society. (Introduced by L. T. Evans.)
- 215. The reproductive behavior of the giant tortoises, *T. vicina* and *T. vanderburghii*. L. T. Evans, New York Zoological Society.
- 216. The visual learning of *Testudo vicina*. John V. Quaranta and L. T. Evans, New York Zoological Society. (Introduced by C. C. Little.)
- 217. Differences in the pattern of sexual behavior of low-drive and high-drive male guinea pigs. William C. Young, University of Kansas.
- 218. Strength of the sex drive in thyroxine-injected male guinea pigs. William C. Young, University of Kansas.
- 219. Vocality, as a factor in the ecology of the alligator. L. T. Evans and J. Quaranta, New York Zoological Society.

Cellular Physiology

- 220. Glycerophosphatases in the frog kidney. Thomas J. King and Ross F. Nigrelli, Washington Square College, and the New York Zoological Society.
- 221. The effect of DDT on the activity of phosphatases in various tissues of the chicken. Harold Burlington, Syracuse University. (Introduced by V. F. Lindeman.)
- 222. The rate of glycolysis and ATPase activity in the developing chick retina. V. F. Lindeman, Syracuse University.
- 223. Carbon dioxide requirements of the early chick embryo. Nelson T. Spratt, Jr. The Johns Hopkins University.
- 224. Purification of firefly luciferin. William D. McElroy and Bernard L. Strehler, The Johns Hopkins University.
- 225. Reaction of plasma cells in the spleens of rats fed protein deficient diets. Paul G. Roope and Jerry W. Brown, University of Kansas.
- 226. The alkaline phosphatase activities of subcellular particulates. Frances Green Kallman and M. J. Kopac, Department of Biology, Washington Square College.
- 227. The action of cations on the protoplasm of *Amoeba proteus* and *Pelomyxa carolinensis*. Robert Kassel and M. J. Kopac, Washington Square College.
- 228. Temperature-pressure experiments on cell division. Douglas Marsland, New York University and Marine Biological Laboratory.

Cytology

- 229. Effect of thymonucleodepolymerase and acid hydrolysis on methyl green staining of chromatin. Cecile Leuchtenberger, Marion Himes and Arthur W. Pollister, Columbia University.
- 230. The insemination of amphibian body cavity and oviducal eggs. Henry W. Aplington, Jr., Muhlenberg College.
- 231. The effects of carbamates on onion roots. Verne van Breemen, State University of Iowa. (Introduced by Harold W. Beams.)

- 232. Lesion induction in the chorioallantois by suspensions of Rous sarcoma, *Tetrahymena geleii* and various fractions of these cells. R. A. Fennell, Michigan State College.
- 233. Heteroploidy in a "Natural Population" of *Amblystoma punctatum*. Donald P. Costello and Catherine Henley, University of North Carolina.
- 234. The finer structure of the resting chromosome. William Hovanitz, University of San Francisco.

Ecology

- 235. Body temperature of Leach's Petrel. G. Edgar Folk, Jr., Bowdoin College.
- 236. Further study of the pselaphid beetle population of an Illinois prairie. Orlando Park, Stanley Auerbach and Marie Wilson, Northwestern University.
- 237. On cultivation of larvae of *Ostrea lurida*. H. C. Davis, U. S. Department of the Interior, Fish and Wildlife Service. (Introduced by V. L. Loosanoff).
- 238. Vertical distribution of oyster larvae of different ages during the tidal cycle. V. L. Loosanoff, U. S. Department of the Interior, Fish and Wildlife Service.
- 239. Gonad development and spawning of oysters at several constant temperatures. V. L. Loosanoff and H. C. Davis, U. S. Department of the Interior, Fish and Wildlife Service.

Embryology

- 240. The effect of antuitrin S on the ovaries of immature hamsters (*Cricetus auratus*). F. V. Rollins and S. Milton Nabrit, Atlanta University.
- 241. A histological study of forelimb regeneration in *Triturus viridescens*. Lemuel A. Lewie, Jr. and S. Milton Nabrit, Atlanta University.
- 242. The distribution of phosphorus³² in the early chick embryo in the presence of vitamin D. Louis A. Hansborough and Philip Nicholas, Howard University.
- 243. Localization of prospective areas in the blastoderm of the pea beetle *Callosobruchus maculatus* Fabr., as determined by ultraviolet (2537 Å) irradiation injury. Alfred Brauer, University of Kentucky and Oak Ridge National Laboratory.
- 244. Determination of the relationship between the principal egg and larval axes of *Saccoglossus (Dolichoglossus) kowalevskyi*. Arthur L. Colwin and Laura Hunter Colwin, Queens College.
- 245. The modification of developmental pattern in the sand dollar with sodium azide. Olin Rulon, Northwestern University.
- 246. The modification of developmental pattern in the sand dollar with thiourea. Olin Rulon, Northwestern University.
- 247. Localization of hemoglobin-forming areas in the early chick blastoderm cultivated *in vitro*. George W. Settle, The Johns Hopkins University. (Introduced by Mary E. Rawles.)
- 248. Can prospective pigment cells of the chick embryo be identified by means of vital dyes during the course of their migration into the skin and feather germs? John W. Saunders, Jr., The Johns Hopkins University and the University of Chicago.

- 249. The development of spinal ganglia following the amputation of hind limb buds in *Rana pipiens* larvae. James W. Merritt, State University of Iowa. (Introduced by Jerry J. Kollros.)
- 250. Nutritional requirements of the early chick embryo. III. The metabolic basis of morphogenesis and differentiation as revealed by the use of inhibitors. Nelson T. Spratt, Jr., The Johns Hopkins University.
- 251. Carbon dioxide requirements of the early chick embryo. Nelson T. Spratt, Jr., The Johns Hopkins University.
- 252. The cumulative effect of oxygen-pressure in the blocking of gastrulation in the embryo of *Rana pipiens*. Olin E. Nelson, University of Pennsylvania.
- 253. Connections between cerebral cells and thigh muscles in their simultaneous brephoplastic intraocular transplantation in the mouse. Raoul Michel May, University of Paris.
- 254. Development of portions of 72- and 96-hour avian otocysts in chorioallantoic grafts. Edythe M. Nagler and Hiram J. Evans, Syracuse University.
- 255. The effect of sodium succinate on the development of succinic dehydrogenase in *Amblystoma punctatum*. E. J. Boell, Yale University.
- 256. Development of cholinesterase in the central nervous system of *Amblystoma punctatum*. E. J. Boell and S. C. Shen, Yale University.
- 257. Pituitary studies in the golden hamster, *Cricetus auratus*: I. Normal development and cellular differentiation. Valeria M. Krol, University of Chicago. (Introduced by Carl R. Moore.)

Endocrinology

- 258. Histochemical evidence of early differentiation of the suprarenal gland of the chicken. Alden B. Dawson, Harvard University.
- 259. Partial maintenance of the adrenal cortex by intraocular grafts of anterior pituitary tissue in the adult guinea pig. Malvina Schweizer and Margaret E. Long, Washington Square College.
- 260. The normal blood picture of the young alligator (*Alligator mississippiensis*) with observations on hematological effect of thiourea injection. Philip H. Lieberman and Harry A. Charipper, Washington Square College.
- 261. Further studies on the relation between wakefulness and the gonadal cycle in birds. Albert Wolfson, Northwestern University.
- 262. Serum uptake of radio-calcium in guinea pigs. R. V. Talmage, J. W. Beck, and E. T. Adams, Rice Institute.
- 263. Opposing actions of pitressin and cortical extract in hypophysectomized rats. W. J. Eversole and C. M. Osborn, Syracuse University.
- 264. Temperature studies in dwarf mice. D. H. Schonholz and C. M. Osborn, Syracuse University.
- 265. The effects of the three methyl ether of bis-dehydrodoisynolic acid (MDDA) on the reproductive system of *Rana pipiens*. Alfred A. Renzi and Hiram J. Evans, Syracuse University.

266. Studies on the effectiveness of desoxycorticosterone acetate (DCA) replacement therapy in adrenalectomized rats fed diets high in protein but free of carbohydrates. Elizabeth F. Place and W. J. Eversole, Syracuse University.
267. Properties of the antidiuretic substance in the serum of normal rats. James H. Birnie and W. R. Boss, Syracuse University. (Introduced by Robert Gaunt.)
268. Intraocular homoplastic transplantation of the anterior lobe of the frog pituitary. Robert F. Kallman, Albert S. Gordon and Harry A. Charipper, Washington Square College.

Genetics

269. Weight variation between piebald and normally colored hamsters. Charles L. Foote and Harrison E. Bullock, Southern Illinois University.
270. Parallel distribution of gene frequencies in seven species of *Colias* butterflies. William Hovanitz, University of San Francisco.

General Morphology

271. The Oonopidae of Panama. A. M. Chickering, Albion College.
272. Growth of the kidneys in the fetal dog. Homer B. Latimer, University of Kansas.
273. Variations in the number per litter and in the individual weights in each litter, in a series of dog fetuses. Homer B. Latimer, University of Kansas.
274. Sexual dimorphism in the anal fin and anal fin suspensorium of *Anableps anableps*. C. L. Turner, Northwestern University.
275. The morphology and histology of the pancreas of the platyfish, *Platyopocilus maculatus*. Gladys Reiter and Ross F. Nigrelli, Washington Square College.

General Physiology

276. The effect of small doses of gamma radiation on the respiration and development of the prediapause eggs of grasshoppers. Theodore N. Tahmisian and Jeanne D. Gasvoda, Argonne National Laboratory.
277. Fixation of $C^{14}O_2$ in grasshopper eggs. Theodore N. Tahmisian and Donald E. Buchanan, Argonne National Laboratory.
278. The effect of B irradiation on the respiration and morphology of *Melanoplus differentialis* embryos. Theodore N. Tahmisian and Jeanne Gasvoda, Argonne National Laboratory.
279. The effect of pH on the sodium azide and sodium cyanide inhibition of respiration in *Oryzias latipes*. Margwyn Harris and S. Milton Nabrit, Atlanta University.
280. Fatty acid and protein synthesis in *Chilomonas paramecium*. B. J. Sullivan, Boston College. (Introduced by Sr. Elizabeth Seton MacDonald.)
281. Mechanism of action of antihistaminic drugs. T. C. Barnes, Hahnemann Medical College.
282. Respiration and weight changes in *Phormia* larvae after exposure to DDT. John B. Buck, Margaret L. Keister and Irene Posner, National Institutes of Health.
283. Culture of mantle tissue of several species of marine molluscs. Gerrit Bevelander and Josephine Martin, New York University.

284. Distribution of free amino acids in nerve tissue. Eugene Roberts and Pinckney J. Harman, Washington University School of Medicine, New York University College of Medicine, and Jackson Memorial Laboratory.
285. Responses of the red chromatophores of the fiddler crab. Frank A. Brown, Jr., Muriel I. Sandeen and H. Marguerite Webb, Cresap Biological Laboratory, Northwestern University and Marine Biological Laboratory.
286. A persistent rhythmicity in *Uca* red chromatophores. Frank A. Brown, Jr., H. Marguerite Webb and J. Bruce Guyselman, Cresap Biological Laboratory, Northwestern University, and Marine Biological Laboratory.
287. Black chromatophores of *Uca* as independent effectors. Frank A. Brown, Jr., J. Bruce Guyselman and Muriel Sandeen, Northwestern University and Marine Biological Laboratory.
288. Vagal tonicity in the turtle. Jacob E. Wiebers, Betty Blue and William A. Hiestand, Purdue University.
289. The effect of inanition and food and water deprivation on the survival time at low temperatures in the albino mouse. Nathan Primack and William A. Hiestand, Purdue University.
290. The effects of *veratrum viride* on the blood sugar level of the albino rat. David E. Mann, Jr., Arthur G. Zupko, Philip V. Hammond and William T. Rockhold, Purdue University. (Introduced by William A. Hiestand.)
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292. On some problems concerning the life cycle of mycetozoa. P. H. Gehenio and B. J. Luyet, Biodynamics Research Laboratory and St. Louis University.
293. A study of the glucose content of the blood of *Pomarine jaegers* from the Point Barrow region in Alaska. X. J. Musacchia, St. Louis University; Bernard J. Sullivan, Boston College, and Louis A. Susca, New York Medical College. (Introduced by Secretary.)
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295. Sugar in the body fluid and tissues of a sea-urchin. Schuyler V. Hilts and Arthur C. Giese, Stanford University.
296. Ultraviolet resistant bacteria in albumen solutions. Arthur C. Giese, Stanford University.
297. The effect of thiamin deficiency upon the histology of the thyroid of the albino mouse. Norma Vincent and J. Theron Illick, Syracuse University.
298. On the presence in the sera of domestic fowl of naturally-occurring "agglutinins" for certain melanins. James D. Ebert and S. H. Goodgal, The Johns Hopkins University. (Introduced by B. H. Willier.)
299. Change in rate of growth initiation on season. Frederick S. Hammett, The Lankenau Hospital Research Institute, Philadelphia.
300. The antagonistic action of adrenal cortical extract on the inhibitory metabolic effect of large doses of sesame oil. Thomas B. Calhoon and Clifford A. Angerer, Ohio State University.
301. The effect of x-irradiation on the respiratory metabolism of non-nucleated erythrocytes in vitro. Luis Angelone, Joseph L. Morton and Clifford A. Angerer, Ohio State University.

302. The interaction of dilute amyl carbamate and certain cations on the resting potential of frog nerve. Frederick Crescitelli and Philip B. Hollander, University of California, Los Angeles.
303. Quantitative evaluation of histochemical preparations for phosphatases. W. L. Doyle, University of Chicago.
304. Effect of salt concentration on histochemical phosphatase preparations. W. L. Doyle, University of Chicago.
305. Tumor mortality and sex in *Leucophaea maderae* (Orthoptera). Berta Scharrer, University of Colorado.
306. Fat metabolism in tumor bearing insects *Leucophaea maderae* (Orthoptera). Virginia T. Wilson and Berta Scharrer, University of Colorado.
307. The electrical response to illumination of the median ocellus of *Limulus*. Verner J. Wulff, Jr., University of Illinois.
308. Histological changes induced in the rat by feeding on acid hydrolyzed tryptophan-deficient diet. F. B. Adamstone, University of Illinois and Harry Spector, Quartermaster Food and Container Institute for the Armed Forces.
309. Some aspects of behavior of oysters accustomed to different salinities. V. L. Loosanoff and P. B. Smith, Department of the Interior, Fish and Wildlife Service.
310. Increased incidence of a tumor of *Drosophila* in the presence of high concentrations of arginine. Louise Palmer Wilson, Wellesley College.
311. Effect of decompression on the glucose concentration of the blood of the frog, *Rana pipiens*. Robert K. Houlihan and John J. Petronio, Boston College.

Parasitology

312. Parasites of the Ranidae (Amphibia). XV. A. C. Walton, Knox College.
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315. The effects of x-ray on *Rhabditis* species. Lyell J. Thomas, University of Illinois.

Protozoology

316. The use of uroid as a taxonomic criterion for certain amebas. Eugene C. Bovee, University of California at Los Angeles. (Introduced by T. L. Jahn.)
317. Some observations concerning the morphology, locomotion and rate of activity of *Trichamoeba osseosaccus* (Schaeffer). Eugene C. Bovee, University of California at Los Angeles. (Introduced by T. L. Jahn.)
318. Observations on *Eimeria mohavensis* sp. nov. from the Kangaroo Rat, *Dipodomys mohavensis*. David J. Doran and Theodore L. Jahn, University of California at Los Angeles.
319. Studies of amphibian trichomonads in culture. Robert Samuels, University of California at Berkeley. (Introduced by Harold Kirby.)
320. The effects of colchicine on a flagellate protozoan, *Trichomonas augusta*. Robert Samuels, University of California at Berkeley. (Introduced by Harold Kirby.)

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ABSTRACTS

PAPERS PRESENTED IN PERSON

General Physiology (Section A)

5. THE METABOLISM OF STARVED NYMPHS OF THE GRASSHOPPER, *CHORTOPHAGA VIRIDIFASCIATA*. Daniel Ludwig, New York University, University Heights. (Lantern; 15 min.)

Determinations were made of the carbohydrate, fat, and nitrogen content of normal grasshopper nymphs and of others starved for one week or until death in desiccators over a saturated solution of ZnSO_4 (relative humidity, 82%). Previous experiments (Ludwig, 1937) had shown that during exposure to this humidity, the water content of the insect remained constant. At the end of one week, the glycogen content had dropped from a normal value of 1.71% to 0.06%. Corresponding percentages for glucose were 1.31 to 0.55; for free fat, 4.04 to 3.93; for nitrogen 2.82 to 3.70. The average time of survival was 15.4 days. At the time of death, the percentage values were as follows: glycogen 0.08; glucose, 0.53; fat, 2.13; and nitrogen, 4.85. These figures show that all of the available carbohydrate is used during the first week, and that fat serves as the principal source of energy during the remainder of the survival period. The nitrogen content shows an apparent increase because of the loss of weight which accompanies starvation. However, after the first two days of inanition, there is a small constant excretion of nitrogen throughout the survival period, the total loss amounting to approximately 10% of the nitrogen originally present.

6. THE EFFECT OF TEMPERATURE ON THE GROWTH AND OXYGEN CONSUMPTION OF THE CHICK EMBRYO. Donald Greiff and Henry Pinkerton, St. Louis University. (Lantern; 15 min.)

Embryonate eggs were incubated at 37.5° for 8 days, at which time oxygen consumption of the complete embryo (embryo plus membranes) and the dry weight of the embryo proper were determined. Oxygen consumption was determined by placing 20 eggs in a sealed container, immersing the container in a water bath maintained at the temperature of incubation, removing samples of air from the containers at known time intervals, and analyzing these air samples in a Haldane-Henderson Gas Analyzer. On removal from the respirometer, the embryos (membranes removed) were dried to a constant weight at 110°C .

After the preliminary incubation period, the embryonate eggs were divided into groups and incubated at 34°C ., 36°C ., 37.5°C ., 39°C ., 40°C . and 41°C . for a period of 5 days. Oxygen consumption and dry embryo weight were then determined. At the end of the preliminary incubation period, the mean dry weight per embryo was 65 mg and the mean oxygen uptake was 0.38 cc of oxy-

gen per egg per hour. After 5 days at 34°C. the mean weight was 350 mg and mean oxygen consumption was 5.23 cc of oxygen per egg per hour; at 36°C. mean weight was 549 mg and mean oxygen uptake 8.75 cc; at 37.5°C. mean weight was 662 mg and mean oxygen uptake 10.81; at 39°C. mean weight was 708 mg and oxygen utilization 11.53 cc; at 40°C. mean weight was 724 mg and oxygen consumption 10.81 cc; and at 41°C. mean weight was 736 mg and oxygen consumption 7.45 cc.

In eggs incubated at 41°C. large blebs filled with a viscous fluid were formed in the choriollantoic membrane, and the dramatic decrease in oxygen uptake at this temperature may be due to these blebs acting as a mechanical barrier to oxygen diffusion. The continued growth of the embryo may indicate a shift in the type of metabolism.

7. A TEMPERATURE DIFFERENTIATION OF THE DUAL ACTION OF AMYL CARBAMATE ON FROG NERVE. Frederick Crescitelli (with the technical assistance of Philip B. Hollander and Alice J. Olmstead), University of California, Los Angeles. (Lantern; 15 min.)

The results of this investigation confirm and extend the concept of a dual action by the carbamate molecule as expressed earlier (Crescitelli, 1948). In the present report the rate of hyperpolarization of frog nerve by amyl carbamate is shown to be essentially the same at 5.2°C. and 23.6°C. Recovery from the hyperpolarized state, however, is slower at the lower of these temperatures. In contrast, the depolarization produced with stronger solutions of amyl carbamate is considerably reduced by a change in temperature from 23.6°C. to 5.2°C. This action of temperature suggests two independent processes induced in the nerve by the amyl carbamate. The first, which is associated with electrical positivity, may be a physical action on the membrane; the second, associated with negativity, may be more nearly chemical.

A comparison of the depolarization of frog nerves by the potassium ion and by strong amyl carbamate solutions has revealed a different effect of temperature on these two processes. Whereas the depolarization of K^+ is greater at 5.2°C. than at 23.6°C., the carbamate depolarization is affected in the reverse manner by this temperature change. A different mechanism for depolarization by these two substances is thus indicated.

Some of the results in this paper are discussed in terms of recent experiments and concepts of the Na^+ transport mechanism in nerve fibers, the cat erythrocyte, and the frog skin.

8. ON THE SPAWNING BEHAVIOUR OF OYSTERS AND OF VENUS MERCENARIA WITH ESPECIAL REFERENCE TO THE EFFECTS OF SPERMATIC HORMONES. Thurlow C. Nelson and H. H. Haskin, Rutgers University. (Lantern; 15 min.)

Spawning in *Ostrea* (*Gryphaea*) *virginica*, *O. angulata*, and *O. gigas* and in the quahaug clam, *Venus mercenaria*, is a group reaction stimulated by a hormone carried on the sperm. This hormone, first demonstrated by Galtsoff in *virginica*, is soluble in alcohol and benzene. Oyster sperm carry a second hormone, discovered and named diantlin by Nelson, which is a conjugated protein.

This relaxes the ostia of the gills, aiding in passage of eggs through the ostia in the female and markedly increasing the rate of water passage in the male. The sperm of *Venus* do not bear diantlin. Correlated with this is the passage of eggs out through the excurrent siphon, not through the ostia onto the surface of the demibranchs as in oysters. Female oysters stimulated to spawn by high temperatures in the absence of sperm show large masses of eggs retained within the water tubes associated with failure of the ostia to enlarge in the absence of diantlin. We have thus far been unable to induce spawning in *Venus* in the absence of sperm. Temperatures within which spawning has occurred in our laboratory have ranged from 22.4 to 30°C. We have confirmed the observations of Loosanoff and Davis at Milford, Conn. that quahaugs can be induced to spawn and produce viable larvae in midwinter if gradually warmed over several weeks.

9. SOME REACTIONS OF THE TRACHEAL SYSTEM IN *PHORMIA* LARVAE. John B. Buck and Margaret L. Keister, National Institutes of Health. (15 min.)

When mature *Phormia regina* larvae are submerged in oxygenated water the tracheae collapse in a few minutes. When submersion is not longer than about an hour the tracheae will refill with gas if the larvae are transferred either to air or to aerated water. Experiments on larvae with all spiracles ligated show that the gas leaves and enters the tracheae through the tissues. If exposure is prolonged the tracheae gradually fill with a liquid, usually irreversibly. The liquid contains much material precipitable by trichloroacetic acid, and darkens on exposure to air. A theory to explain the gas exchanges and the origin of the liquid, and a discussion of the bearing of the experiments on tracheal permeability, will be offered.

10. REVERSIBILITY OF TRACHEAL FILLING IN *SCIARA* LARVAE. Margaret L. Keister and John B. Buck, National Institutes of Health. (15 min.)

In a previous study it was shown that freshly molted *Sciara coprophila* larvae have liquid-filled tracheae; that shortly after the molt, gas from an internal source replaces the liquid; and that gas will appear in the tracheae if the larvae are in 99.7% CO, CO₂ or N₂ with 0.3% O₂, but not in total absence of O₂. In searching for factors responsible for initiating the appearance of gas, it was found that larvae which have begun to exhibit the characteristic premolting behavior will fill their new tracheal systems with gas without any rupture of the body cuticle, if kept immobilized in 0.3% O₂. It thus appears that the actual shedding of the exuviae is not the stimulus for initiation of gas-filling. It was further found that premolt larvae which have become gas-filled, as described above, will usually reverse the process when returned to air, becoming partially or completely liquid-filled. Such larvae, if left in air, will then often fill their tracheae a second time with gas, either with or without molting, or if returned to 0.3% O₂ will fill again with gas without molting. The relations of the reversal of gas-filling to the normal process, and to theories of its causation, will be discussed.

11. EXTREME LONGEVITY IN ROTIFERS. A. I. Lansing, Washington University. (Lantern; 15 min.)

Selection experiments with rotifers have produced data which indicate that ageing involves an orderly process of a non-genic nature. Old rotifers transmit through their eggs a capacity for shortening longevity of the F_1 which may be accentuated through a series of generations by repeated selection. The degree of longevity reduction is directly dependent upon the age of the adult parent rotifer. Maturation of old orthoclone rotifers is accelerated.

The capacity for accelerating ageing is not manifested by orthoclones of less than full grown rotifers. An adolescent orthoclone (5 day old) has been carried through 54 generations with some of the animals living more than 100 days (normal life span of 24 days). Maturation of these rotifers is greatly retarded.

The implications of these experiments will be discussed.

12. ISOLATION AND PROPERTIES OF RAT LIVER NUCLEI. Karl M. Wilbur, Norman G. Anderson and Max V. Skeen, Duke University. (Lantern; 10 min.)

Liver nuclei almost completely free of other cell constituents have been prepared by differentially centrifuging a homogenate through a density gradient made by mixing varying proportions of an isosmotic salt solution (I) at pH 7.1 and hyperosmotic sucrose. Solution I consisted of 0.05 M K_2HPO_4 , 0.038 M KH_2PO_4 , and 0.044 M $NaHCO_3$. Isosmotic and hyperosmotic mixtures of solution I and sucrose solutions at 0°C. maintained for several hours approximately 80% of the isolated nuclei in a condition optically indistinguishable from nuclei of freshly isolated whole cells. Under similar conditions pure sucrose solutions were less satisfactory. Addition of 0.002 M $CaCl_2$ to the salt-sucrose mixtures resulted in nuclear shape and an abnormal appearance.

The effects of a wide range of tonicities of sucrose and sucrose salt mixtures on the volume of isolated nuclei was studied using a photographic technique. The volume changes were relatively small in all solutions examined.

13. THE RELATIONSHIP BETWEEN CARBONIC ANHYDRASE AND GROWTH IN MOLLUSKS. Norman G. Anderson and Karl M. Wilbur, Duke University. (5 min.)

The carbonic anhydrase activity of *Busycon carica* was found to increase markedly during development within the egg case. The enzyme activity of whole larvae during the early stages of shell formation may be more than 40 times that of larvae just prior to shell deposition. Larvae with shell may show an increase of nearly 20 fold in enzyme activity in growing from a length of 2.0 mm to 3.4 mm.

In the mantle of the adult oyster *Ostrea virginica* the carbonic anhydrase activity was in general inversely proportional to the length of the shell, oysters averaging 4 inches in length having about 25% of the activity of oysters 1½ inches long. The significance of this relationship is not known. Carbonic anhydrase is present in both gametes of the oyster, the concentration being considerably higher in the spermatozoan than in the ovum.

14. ASSAY FOR THE GROWTH AND DIFFERENTIATION HORMONE OF LEPIDOPTERA BY THE METHOD OF TISSUE CULTURE. Edmond L. Schmidt and Carroll M. Williams,¹ Harvard University. (Introduced by Henry G. Bigelow.) (Lantern; 15 min.)

Spermatocytes from testes of dormant pupae of the silkworms, *Platysamia cecropia* and *Samia walkeri*, develop promptly into spermatids when placed in a hanging drop of blood obtained from donor silkworms at specific stages. The periods during which the blood is active in this respect correspond roughly to those periods during which the prothoracic glands are thought to secrete the "growth and differentiation" hormone which provokes the metamorphosis of the intact animal. The differentiation of the spermatocytes appears to be an exceptionally sensitive test for the presence of this hormone. Using this test, the hormone is found to be absent from the mature larva prior to the stage when the inner coat of the cocoon is spun. It then appears abruptly in the blood and persists until some time after pupation, apparently in low titer during the later part of this period. It is virtually absent from diapausing pupae and from chilled pupae, but reappears in the pupal blood when adult development is initiated. The hormone is then present throughout adult development, and persists in the newly emerged adult in sufficient concentration to cause growth of spermatocytes *in vitro*. This method of assay is being utilized in further studies of the occurrence and properties of the growth and differentiation hormone.

¹ This study was aided by the Lalor Foundation and the American Cancer Society.

15. EXTRACELLULAR FLUID VOLUME OF THE RAT,¹ R. V. Talmage, R. C. Frost, R. J. Shalek, J. H. Burr and F. A. Garrett, The Rice Institute. (15 min.)

A modification of the isotope dilution method for the determination of apparent extracellular fluid volume is here presented in which it has been determined that this volume in rats weighing 250 gm is $28.6\% \pm 0.206$ of the body weight. This technique is based on the values obtained from the measurement of activity of 4, 20 cmm samples of blood serum taken from the tail of the rat, the blood being obtained at stated intervals after the intraperitoneal injection of Na²². The method is of sufficient accuracy so that it can be shown that the apparent extracellular fluid volume of the rat varies inversely as a linear function of the body weight. Thus, in a male rat weighing 220 gm this volume is 29.3% of body weight, that of a 280 gm male is only 27.9. This percentage regression is small enough, however, that it is possible to determine the fluid volume in rats weighing between 200 and 300 gm with a standard error of rarely over 1%, by utilizing 8 rats with a weight range of as much as 80 gm. The value obtained is that for the extracellular fluid volume of a rat with a weight corresponding to the average weight of the rats used. This method has the further advantage in that since the animals are not sacrificed in the process, it provides a means for studying the effect of test substances on the rate of sodium penetration and excretion and for studying shifts in apparent extracellular fluid volume in a single animal.

¹ Aided by a grant from the United States Atomic Energy Commission.

16. ANTIBIOTIC EFFECTS OF FOWL LEUCOCYTES IN VITRO. C. G. Grand, Washington Square College of Arts and Science, New York University, New York. (Introduced by Robert Chambers.) (Lantern; 10 min.)

The antibiotic substance present in leucocytes of the fowl was demonstrated by culturing chicken buffy coat and chick embryonic spleen in the presence of bacteria (*Staphylococcus aureus*). Broth suspensions of the bacteria were mixed in the culture medium at the time the cultures were prepared. Six hours after incubation and, when a large number of leucocytes had migrated out of the fragment, a zone of about 1 to 1.5 mm in width surrounding the explant was free of bacterial colonies although no leucocytes were present in this zone. Beyond this zone the bacterial colonies developed and as the leucocytes migrated further out of the explant, these colonies broke down and disintegrated even before the leucocytes could reach them. When the cultures were fixed and stained with bacterial stain no bacteria could be demonstrated within this zone. This phenomenon was not observed in cultures of other chick tissues or in cultures of human, rabbit, guinea pig, rat and mouse blood buffy coats or spleen.

Embryology (Section B)

17. INHIBITION OF MELANOPHORE MIGRATION IN THE WHITE AXOLOTL. H. Clark Dalton, Carnegie Institution of Washington. (Lantern; 15 min.)

The differentiation of pigment cells from explanted neural folds of black and white axolotls in Holtfreter's solution has shown that potential melanophores of both genotypes were capable of melanin synthesis in vitro and were alike in their capacity for proliferation and migration in tissue culture. No disadvantage for melanin synthesis in white embryos was exhibited by melanophores from grafts of neural folds to the mid-ventral belly region of both strains or from reciprocal homotopic neural crest grafts between the strains. Melanoblasts of both genotypes appeared to migrate freely beneath epidermis of the black strain but not beneath epidermis of the white strain. Melanophores appeared beneath epidermis grafted from black to white embryos, if the graft covered the dorsal part of the flank but not if the graft covered the ventral part of the flank only. The areas of triangular pieces of epidermis grafted from black to white embryos became more densely populated by melanophores when the base of the triangle was dorsal than when the apex was dorsal. These results support the idea that the genetic differences in pigmentation between the two strains are mediated through the tissue environment of the chromatophores, which in the white strain has an inhibitory effect concerned, not with melanin synthesis, but with the migration of melanoblasts.

18. INNERVATION OF FIN TRANSPLANTS IN *OPSANUS TAU*. T. H. Waterman and J. S. Nicholas, Yale University. (Lantern; 15 min.)

The comparative anatomy of deep-sea angler-fishes suggests that the cephalic fishing apparatus, derived from anterior rays of the dorsal fin, may have wide-

spread morphogenetic effects on the cranial region. *Opsanus* belongs to the same order of fishes as these ceraticids but lacks the fishing apparatus, or illicium. Experiments were designed to test the effects of transplanting the spinous dorsal, normally postcranial, forward onto the midline of the skull. Larval fishes, approximately one month after fertilization, between 10 and 20 mm total length were operated and reared in the laboratory for 25 weeks. Serial sections, stained by the Bodian technique, and cleared and alizarin-stained whole preparations were made of unoperated controls and of the 6 successfully operated fish which survived for this period. The grafts, inserted between the skin and the skull, consisted at the end of the experimental period of a mass of tissue largely made up of fin rays and their pterygiophores. Some striated muscle, connective tissue, and aggregation of melanin were also found in the graft region. Furthermore a rich supply of nerve fibers penetrated the fin tissue. Unlike the innervation of the angler-fish illicium, mainly or exclusively spinal in origin, the *Opsanus* graft innervation was of cranial origin. The sensitivity of the graft region in the living operated fish showed that at least part of the innervation was somatic sensory. Further study is required to determine the specific origins of the innervation and to show whether grafts more massive in relation to skull size would have larger morphogenetic effects on cranial components.

19. DEVELOPMENT OF RESPIRATORY ENZYMES IN RAT MUSCLE.¹ S. C. Shen, Osborn Zoological Laboratory, Yale University. (Introduced by A. Petrunkevitch.) (Lantern; 15 min.)

A study was made of the development of cytochrome oxidase, succinic oxidase and succinic dehydrogenase in rat skeletal muscle throughout the period from the 14th day of gestation to the third week after birth.

In saline homogenates of the muscle, the enzyme activity per unit weight tissue nitrogen increases two to three fold during the period investigated. There is however considerable variation among samples due principally to the crudeness of the enzyme preparation. The saline homogenate was accordingly fractionated by differential centrifugation. By far the highest concentration of the enzymes is then found in the fraction consisting of large granules sedimentable at 9,000 g. These granules, characterized by their intense enzyme activity, uniform size and stainability with Janus Green B, are presumably similar to the mitochondria granules isolated from liver cells. Their enzyme activity is highly uniform among different preparations. While the amount of the granules extractable per unit weight of muscle remains practically constant, the Q values (activity per milligram nitrogen per hour) of all three enzymes in the granules increase exponentially with age from the 15th day of gestation to the 12th day after birth, reaching a steady level thereafter. Concomitantly, there is a progressive decline in the ribonucleic acid content of the granules. The results suggest an active synthesis of the respiratory enzymes, probably associated with changes in nucleoproteins, within the mitochondria granules during histogenesis of muscle.

¹Supported in part by a grant from The American Cancer Society through the NRC Committee on Growth.

20. EXPERIMENTAL MODIFICATION OF CHOLINESTERASE DEVELOPMENT IN THE MID-BRAIN OF *AMBLYSTOMA PUNCTATUM*.¹ E. J. Boell and S. C. Shen, Osborn Zoological Laboratory, Yale University. (Lantern; 15 min.)

The growth and differentiation of the mid-brain of *Amblystoma punctatum* has been altered by experimental modification of the development of the eye, and the effects of this on cholinesterase development have been studied. In one group of animals, the right eyes were extirpated at stage 26, before formation of the optic nerves. In another group, the potentially large, fast growing eyes were removed from *Amblystoma tigrinum* embryos at stage 26, and these were transplanted to the right eye sites of *Amblystoma punctatum* hosts of the same stage. For the determinations of enzymatic activity, the mid-brain was divided into 6 regions: left and right anterior dorsal, left and right posterior dorsal, and left and right infundibular.

The results of these experiments show that removal of the right eye from *Amblystoma punctatum* results in hypoplasia of the mid-brain on the left side as evidenced by a somewhat lower total nitrogen content. A corresponding biochemical change is indicated by the failure of cholinesterase activity, per unit weight tissue nitrogen, to develop in the left optic portion to the same extent as in the opposite, or control, side.

Transplantation of an *Amblystoma tigrinum* eye to the right side of an *Amblystoma punctatum* host induces a marked hyperplasia of the mid-brain on the opposite side. Correspondingly, the cholinesterase activity of the optic portion of this side is significantly greater than that of the opposite side.

These experiments suggest that the development of cholinesterase activity in the mid-brain is intimately correlated with morphological and functional differentiation.

¹Supported in part by a grant from The American Cancer Society through the NRC Committee on Growth.

21. BEHAVIOR OF COMPONENTS OF THE EARLY EMBRYO OF THE MOUSE IN CULTURE AND IN THE ANTERIOR CHAMBER OF THE EYE. Clifford Grobstein, National Cancer Institute, National Institutes of Health, Bethesda 14, Maryland. (Lantern; 15 min.)

Egg cylinders from 6- and 7-day pregnant C-strain female mice have been cut into three sections containing: (1) ectoplacental cone and trophectoderm, (2) the embryonic shield proper, and (3) the remaining extra-embryonic area. When placed in Carrel flasks in plasma clot culture (chicken plasma, chick embryo juice, horse serum and Tyrode's solution) the ectoplacental section produces a mass of giant cells such as have been described in cultures of rabbit egg cylinders (Maximow, '25), and which also develop from whole mouse egg cylinders in culture. The embryonic shield cultivated by itself does not yield such cells.

Upon transplantation to the anterior chamber of the eye of young C-strain males the ectoplacental section produces intra-ocular hemorrhage and hypertrophy, as has been described following similar implantation of tubal mouse

eggs (Runner, '47; Fawcett, Wislocki and Waldo, '47), and as occurs when whole 6- or 7-day cylinders are implanted. Giant cells are conspicuous in sections of such eyes. The embryonic shield section has produced no such reaction when implanted into the eye. It grows and undergoes some differentiation but has not produced giant cells.

Removal of the ectoplacental cone and trophectoderm thus permits study of growth and differentiation of the early embryonic shield, both in culture and in the anterior chamber of the eye, without interference by giant cells.

22. THE EFFECTS OF VARIOUS ABNORMAL AGENTS APPLIED TO EARLY DEVELOPMENTAL STAGES OF *HEMICHROMIS BIMACULATA*, AND THEIR THEORETICAL SIGNIFICANCE. Robert S. McEwen, Janet Bloch Briggs and Margaret Shea Gilbert, Oberlin College. (Lantern; 15 min.)

The jewel fish, *Hemichromis bimaculata*, was subjected during cleavage and early gastrular stages to ultraviolet radiation, X rays, high and low temperatures, distilled water and water containing an excess of $MgCl_2$. As a result certain abnormalities were induced which are similar to those occasionally occurring in controls. Cyclopia never appears in this form, and the abnormalities which do appear are the same regardless of the agent employed or the stage treated. It is concluded that this fish has a tendency to vary abnormally in certain directions, and that abnormal environment merely accentuates this tendency. The bearing of this upon various theories of teratology, including particularly that of "developmental arrest" is discussed. On the basis of the results herein described the opinion is expressed that any theory which accounts for specific abnormalities as caused exclusively by the time at which treatment is applied, is untenable as a general explanation of teratological phenomena.

23. STUDIES OF GRAFTED HEADS OF *RANA PIPPIENS*. Jerry J. Kollros, The State University of Iowa. (Lantern; 15 min.)

Heads, or parts of heads, of *Rana pipiens* embryos in stages 17-20 were grafted to the dorsal surface of small *pipiens* tadpoles (stages I-II). Such preparations permit study of brain development in the absence of influences from the spinal cord. In some grafts, shortly after the initial operation, one or both eyes were removed, as were all or large parts of forebrain and hindbrain. The self-differentiating capacities of the midbrain could thus be studied.

Grafts of entire heads display the usual quivering eye movements of the normal tadpole, simultaneous bilateral external rotation of the eyes, and compensatory conjugate movements of the eyes induced by rotation of the body on its long axis. A rhythmic or near-rhythmic respiratory movement may be present. Smaller grafts, lacking the hindbrain and lacking the lower jaw, display only convulsive activities of the basal musculature of the graft (probably gill arch muscles) and of the eye muscles.

The lamination patterns of the midbrain develop normally in the early stages, but tend to be distorted in those older animals which lack a forebrain, or which

show a pronounced hydrocephaly. With hydrocephaly a marked thinning of the midbrain roof may be seen, as well as elimination of certain laminae. The cell number in the peripheral (white) zones of the midbrain roof tends to be normal or supranormal. Mesencephalic V nucleus cells differentiate normally.

24. EVIDENCE FOR STRUCTURAL SPECIFICITY IN BASIC PROTEINS. Charles B. Metz, Mary T. Foley and Joanne E. Donovan,¹ Yale University. (Lantern; 15 min.)

Basic proteins (protamines, histones) react, through salt linkages, with various acidic substances forming precipitates or agglutinates. Such reactions are generally believed to be highly non-specific. However, it is now found that a certain degree of specificity is required of basic proteins for interaction with cell surfaces. Basic proteins from sperm of 11 species (4 echinoderms, 4 molluscs, 2 annelids, 1 teleost) were tested for agglutinating action on sperm of several or all of 12 forms (4 echinoderms, 5 molluscs, 2 annelids, 1 teleost). Ten of the 11 basic proteins agglutinated sperm from at least one species. Sperm from 10 of the 12 species were agglutinated by at least one of the basic proteins. Therefore at least 10 of the 11 basic proteins and 10 of the 12 kinds of sperm had sufficient basic and acidic groups respectively to permit agglutination. However, three of these 10 preparations showed unique combinations of positive and negative reactions. The remaining 7 fell into two groups of two and one group of three. Several of these 7 were tested on only a few kinds of sperm. Further tests may demonstrate individuality in these also. In all cases tested sperm which failed to give agglutination also failed to absorb the basic protein agglutinin for positively reacting sperm. Therefore it is concluded that failure to agglutinate sperm indicates inability of the basic protein to combine with sperm. The qualitative individual differences in the ability of various basic proteins to combine with sperm are ascribed to some structural specificity in the individual basic proteins.

¹ Aided by a grant from the National Institute of Health, U. S. Public Health Service.

25. AN ANALYSIS OF THE EFFECTS OF CERTAIN ANTI-ORGAN SERA ON THE DEVELOPMENT OF THE EARLY CHICK BLASTODERM IN VITRO. James D. Ebert, The Johns Hopkins University. (Introduced by B. H. Willier.) (Lantern; 15 min.)

Studies of several hundred whole chick blastoderms explanted on a medium of albumen extract + saline agar containing graded amounts of anti-chicken heart or anti-chicken spleen serum, coupled with the use of the precipitin technique, indicate the presence in the blastoderm, as early as the primitive streak stage, of at least one antigen common to both adult chicken heart and spleen. Moreover, antisera display a growth inhibitory capacity not readily attributable to organ-specific properties.

Two groups of albino rabbits were injected with sterile, fresh, saline extracts of adult chicken heart and adult chicken spleen, respectively. Antisera recovered following the primary course of injections had low titers as measured by the

precipitin reaction. Therefore the anamnestic reaction was used to produce workable titers (effective serum dilution 1:256 to 1:512). Antisera produced in this manner were species-specific, but only partially organ-specific. Following absorption with chicken red cells and non-homologous organ extracts, antisera displayed a high degree of organ-specificity.

Definitive primitive streak and head process stages explanted on media containing either absorbed anti-organ serum in final concentrations under 1:80 rapidly undergo disorganization, characterized by (1) disappearance of gross structural features and (2) cell clumping. Explanted head fold and 1-6 somite stages are similarly affected; at these stages, moreover, there is evidence of an anterior-posterior gradient of susceptibility to the antiserum. At dilutions between 1:80 and 1:180, normal morphogenesis occurs in the almost complete absence of growth as determined by study of camera lucida drawings. Development on media containing normal rabbit serum does not differ from that on albumen extract-saline agar alone.

26. DETERMINATION OF STRUCTURAL PATTERNS IN THE SPINAL CORD OF THE CHICK EMBRYO STUDIED BY TRANSPLANTATIONS BETWEEN BRACHIAL AND ADJACENT LEVELS. Byron S. Wenger, Washington University. (Introduced by Viktor Hamburger.) (Lantern; 15 min.)

Transplantation of neural tube between brachial and adjacent levels has been performed in chick embryos of two days' incubation (13 to 25 somites). These grafts undergo excellent histological and morphological differentiation and innervate adjacent regions of the host.

In all cases investigated, cell groups characteristic of a given level are found in grafts from that level and always in the same relative positions, no matter to what host level these grafts were transplanted.

Nerves arising from cervical or thoracic spinal cord, while decidedly more slender than brachial nerves, can form a completely normal brachial plexus pattern under the influence of the normal brachial periphery. Nerves from brachial spinal cord grafted to another level may produce nerve patterns typical for that level (thoracic) without forming a plexus. They do, however, demonstrate a particularly high capacity for growth and frequently make abnormal plexus-like anastomoses.

It is concluded that: (1) As early as they can be identified (by adjacent somites), the cervical, brachial, and thoracic levels of the chick spinal cord are determined with respect to recognizable cell types and patterns of cellular arrangement. (2) Plexus patterns as well as patterns of peripheral distribution are imposed upon nerves by surrounding tissues, but the realization of the final pattern depends also upon factors inherent in the nervous tissue.

27. X-RAY INDUCED DEVELOPMENTAL ABNORMALITIES IN THE MOUSE AND THEIR USE IN THE ANALYSIS OF EMBRYOLOGICAL PATTERNS. Liane Brauch Russell, Oak Ridge National Laboratory. (Introduced by William L. Russell.) (Lantern; 15 min.)

Four hundred and twenty mice, irradiated with 250 kvp X-rays during intra-uterine life, were studied externally and skeletally as newborns. Treated stages

differed by 24-hour intervals and ranged from day $\frac{1}{2}$ to day $13\frac{1}{2}$ after fertilization. Dosages employed were 200r (100r in a few cases) for a survey of all stages; 300r and 400r for dosage comparisons at the last 5 stages. Irradiation before implantation (cleavage stages) increases early pre-natal mortality; those animals surviving to term, however, are normal in 98% of the cases. Irradiation at post-implantation stages permits survival to term, but produces a high incidence of abnormalities characteristic of stage treated and of dose (e.g., 100% posterior shift of thoraco-lumbar border in the $8\frac{1}{2}$ day-200r group, 100% of different specific types of foot abnormality in each of 5 stage-dose groups, etc.). The selective response of structures is presumably due to intrinsic patterns of sensitivity. Critical periods in several developmental processes have been established. Some of these periods are short (e.g., certain parts of the appendicular skeleton are affected at only one stage), others extend over several days (e.g., the tail is sensitive throughout 6 successive stages). Increase in incidence of abnormality with increase in dose indicates that, even where there is an all-or-none effect (actually rarer than graded response), animals at a particular stage are continuously distributed with regard to sensitivity. There are both close similarities and striking differences between radiation induced abnormalities and the effects of known genes.

Cytology (Section C)

28. AN EVALUATION OF AUTORADIOGRAPHS OF BULL SPERM. David W. Bishop, University of Massachusetts. (Lantern; 10 min.)

The autoradiograph technique involving the isotope P^{32} has been applied to the investigation of phosphorus localization in the metabolism of bull sperm. Results are variable depending on the metabolic state of the cells at the time of exposure and are consistent with data obtained with the Geiger Muller counter and biochemical procedures. The posterior portion of the head and the adjacent mid-region tend to accumulate sufficient isotope to energize the emulsion of NTB plates in these areas. Limitations in the method as a functional cytochemical test are inherent in (1) the total amount of P^{32} pick-up which may be inadequate to produce satisfactory autoradiographs and (2) the confusion resulting from the unexpected staining of the sperm themselves by the materials used in the developing technique. The nature of these staining reactions is being further investigated.

29. STUDIES ON THE HISTOCHEMICAL LOCALIZATION OF CHOLINESTERASE IN NERVE TISSUE OF THE DOG.¹ Walter L. Hard and Alvin C. Peterson, Department of Anatomy, School of Medicine, University of South Dakota. (Lantern; 12 min.)

Representative tissues from both central and peripheral nervous systems were treated with the Gomori ('49) technic which purports to show sites of cholinesterase activity. The reactions were essentially equivalent both in reference to intensity and localization when either myristoyl or palmitoyl choline chlorides were used. Lauroyl and stearoyl choline chlorides were not hydrolyzed in visible amounts. In general, the predominant sites of enzyme activity were noted in extra-neuronal tissue immediately associated with nerve cells. Thus, intense

reactions were obtained in brain stem nuclei and cell columns of the spinal cord. The reaction appears to be in glial tissue, since it is outside of the nerve cell, but the nature of the reaction does not permit certain identification of this point. If the localization is not within the glial cell, it must be presumed the enzyme is free within this tissue. The capsular cells, and supporting endoneurium, at the periphery of nerve cells in both dorsal root and sympathetic ganglia exhibit an intense reaction. Nerve fibers in both central fasciculi as well as in peripheral nerve are essentially negative although nuclei of glial and connective tissue cells are reactive. There is a limited and variable enzyme response within the cytoplasm of the nerve cells and the cell nuclei are quite reactive. Experiments designed to injure the nerve cells in selected regions, such as cord and ganglia, have demonstrated that a normal functioning nerve is responsible for the maintenance of the enzyme reaction as herein reported.

¹ This work has been supported by a contract from the Office of Naval Research.

30. AN EXPERIMENTAL STUDY OF SOMATIC REDUCTION IN THE MOSQUITO. Joseph E. Schuh, Fordham University. (Introduced by Charles A. Berger.) (Lantern, 7½ min.)

Somatic reduction divisions occurring during metamorphosis of the larval ileum of mosquitoes was first reported by Berger at the AAAS meeting in 1936. Confirmation of this finding was published by Grell in 1946. Because of the uniqueness of the phenomena involved further experimental confirmation was sought by treating the ileum with colchicine during its metamorphosis. The anticipated effects of this treatment are: first-division multiple complex cells should have their reduction division inhibited and should remain large, highly polyploid cells; later-division cells should show supercontracted *chromatids* instead of c-mitotic chromosomes. The results of these experiments to date are presented.

31. A STUDY OF THE LAMPBRUSH CHROMOSOMES OF SOME SALAMANDERS. M. A. Fontanella, Fordham University. (Introduced by Charles A. Berger.) (Lantern, 7½ min.)

A study was made of the early meiotic stages in oogenesis of *Triturus viridescens* and *Necturus maculosus*. Leptotene, zygotene and pachytene stages are normal. During diplotene there is a tremendous increase in the size of the germinal vesicle and the chromosomes take on the lampbrush characteristics. The question of the polyploid nature of the germinal vesicle at this time is considered. In late diplotene and early diakinesis the lampbrush chromosomes disintegrate in whole or in part and a haploid complement of normal diakinetik bivalents is present. The origin of these diakinetik bivalents is discussed.

32. CHROMOSOME STRUCTURE AS REVEALED BY TRYPTIC DIGESTION. Berwind P. Kaufmann, Carnegie Institution of Washington, and Robert C. MacDuffee, Army Medical Department Research and Graduate School. (Lantern; 15 min.)

The action of trypsin in digesting cellular materials can be resolved into several component phases (Kaufmann, Gay, and McDonald, Cold Spring Harbor

Symp. Quant. Biol. 14, 1949). Treatment of fixed cells of salivary glands of *Chironomus* or *Drosophila* with an aqueous solution of purified trypsin at pH 6 produces no visible alteration of structure. Subsequent short treatment with electrolytes (e.g., 0.05 M phosphate buffer or 0.01 M sodium chloride) and then water produces marked and rapid swelling of the cell with consequent deformation of the chromosomes. Treatment with electrolytes followed by digestion with trypsin does not produce any detectable effect. The extent of nuclear distention obtained with different types and concentrations of electrolytes has been determined by direct measurement, using the phase-contact microscope. In the process of distention, the compact bands of the chromosome become separated laterally into a series of component chromomeres. Additional information about the pattern of organization has been obtained by preparing electron micrographs of the chromosomes in the expanded state, using the methods developed by Schultz, MacDuffee, and Anderson (Science 110: 5, 1949).

33. THE EFFECT OF VISIBLE LIGHT ON THE SURVIVAL OF ULTRA-VIOLET TREATED MICROCONIDIA OF *NEUROSPORA CRASSA*. Sol H. Goodgal, The Johns Hopkins University. (Introduced by Mary E. Rawles.) (Lantern; 15 min.)

The phenomenon of photoreactivation, in which cells ordinarily killed by ultraviolet are reactivated by post-treatment with visible light, appears to be general among the fungi. Treatment of microconidia of *Neurospora crassa* with ultra-violet at a dosage of 120,000 ergs/cm² results in 99.99% killing. When this treatment is followed by visible light of high intensity, viability is increased to 45-50%. The number of conidia photoreactivated is directly proportional to the intensity and the duration of exposure to visible light. The percentage of conidia photoreactivated decreases with delay in the application of the visible light up to a critical time, after which no photoreactivation is obtained irrespective of light intensity. The effect of temperature on the photoreactivation process has also been studied. If conidia treated with ultra-violet are immediately exposed to visible light, it is found that the activation energy for photoreactivation is approximately 8500 calories. However, if the light treatment is delayed, approaching the critical time, the activation energy is approximately 20,000 calories. With increasing doses of ultra-violet, the maximum amount of photoreactivation obtainable decreases. This fact suggests that killing may be due either to (1) the irreversible destruction of essential cytoplasmic or nuclear components or to (2) alteration of substances which may be rendered non-lethal by visible light.

34. EFFECTS OF CARBON DIOXIDE AND NITROGEN GAS ON MITOSIS AS OBSERVED IN LIVING NEUROBLASTS OF GRASSHOPPER EMBRYO. Mary Esther Gaulden, J. Gordon Carlson and Samuel R. Tipton, Department of Zoology and Entomology, University of Tennessee and Biology Division, Oak Ridge National Laboratory. (Introduced by Maurice Whittinghill.) (Lantern; 15 min.)

Mitotic abnormalities resulting from growth of cells *in vitro* in atmospheres of carbon dioxide and nitrogen have been studied for comparison with abnormalities resulting when cells are grown with little or no air. Carbon dioxide

causes the chromatin of all stages of mitosis to become highly refractive, and in the case of middle and late prophase the state of the chromatin "reverts" to a condition resembling earlier stages of mitosis. Cells treated in prometaphase develop small spindles; those treated in metaphase suffer a reduction in the size of the spindle. The duration of these stages is greatly prolonged. All progress of cells in anaphase at the beginning of treatment is stopped for 20-30 minutes, after which time the cells gradually go into telophase. The only observed effects of nitrogen are a "reversion" of middle and late prophase chromatin to a state resembling earlier mitotic nuclei and a slight retardation of metaphase. When cells are grown in a drop culture medium about which there is no air space, the resulting effects are the same as those produced by carbon dioxide treatment. In the case of all three treatments the cells are able to recover rather quickly when air is introduced into the preparations. The fact that cells are more sensitive to an excess of carbon dioxide than to a deficiency of oxygen may indicate that carbon dioxide poisoning is a more effective factor than anoxia in the production of mitotic abnormalities in small, sealed hanging-drop preparations.

35. ELECTRON MICROSCOPE STUDIES ON THE STRUCTURE OF CARDIAC MUSCLE. H. W. Beams, T. C. Evans, C. D. Janney and W. W. Baker, State University of Iowa. (Lantern; 10 min.)

Electron micrographs of guinea pig cardiac myofibrils show all the bands and membranes previously described for cardiac muscle. These include the Z, J, N, E, Q, H and M. In addition, an X band composed of two lines on either side of the M membrane is described. Metafibrils or small bundles of myosin filaments course without apparent interruption through the consecutive J and Q bands. The Z membrane appears to be continuous across the myofibrils and inter-myofibrillar spaces. It apparently aids in holding the myofibrils together.

The intercalated disc is also shown. Electron micrographs of certain other heart components are illustrated. These have been tentatively interpreted as units of Purkinje, nerve and connective tissue fibers.

36. THE DESOXYPENTOSE NUCLEIC ACID CONTENT OF ANIMAL NUCLEI. Hewson H. Swift, Columbia University. (Introduced by A. W. Pollister.) (15 min.)

Photometric determinations were made of individual Feulgen-stained nuclei of several animal species as an indication of the amount of desoxyribose nucleic acid (DNA). Measurements of 11 differentiated mouse tissues and 6 frog tissues support the view of Boivin and Vendrely that the DNA content is identical in all nuclei of an organism. The following exceptions to this rule were found: (a) in mouse, frog and Ambystoma liver, in mouse pancreas, thymus and testis, and in grasshopper testis sheath, nuclei with 2, 4 and 8 times the common value occur; (b) spermatid nuclei contain one-half the normal value; (c) in two dividing tissues (mouse embryonic liver and Ambystoma pronephros) values for interphase nuclei scatter between normal and twice the normal amount. These last data suggest that in these rapidly dividing tissues DNA is accumulated during interphase, before the visible onset of mitosis, and that no further DNA synthesis occurs during mitosis. Sex cells of the mouse testis were studied

from birth to maturity. At birth two classes of premeiotic cells are present, one with the common class of DNA (Class I) and another with twice this value (Class II). At maturity Class II nuclei become the prevalent type, and these are the nuclei which proceed into leptotene. All gonial divisions measured were Class I cells; Class II nuclei and primary spermatocytes contain identical amounts of DNA; secondary spermatocytes have the Class I amount, while spermatids have one-half of Class I. Thus it appears that all the DNA for 4 spermatozoa is present before leptotene.

37. SOME FACTORS INFLUENCING TUMOR INCIDENCE IN *DROSOPHILA MELANOGASTER*. Ernest W. Hartung, Rhode Island State College. (15 min.)

So-called benign tumors appearing in certain strains of *Drosophila melanogaster* are studied genetically, cytologically, and physiologically. In at least two strains the appearance of tumors depends upon a Mendelian recessive factor located on chromosome two, and for one of these the approximate locus has been determined. Studies including the effects of temperature, X-radiation, and quality and quantity of diet, indicate that environmental agencies may affect tumor incidence dramatically in strains showing spontaneous incidence. However, in so far as investigation has progressed, it would appear that they are generally not effective in causing tumors in non-tumorous strains, or in suppressing completely the appearance of tumors in strains normally showing these growths as a spontaneous phenomenon.

38. HUMAN FETAL BRAIN CELLS, 8 DAYS AFTER DEATH, GROWN IN TISSUE CULTURES. Mary Jane Hogue, Medical School of the University of Pennsylvania. (Lantern; 10 min.)

A human fetus measuring 88 mm CR was obtained from a therapeutic abortion. It was kept in an icebox at 8°C. On the eighth day after death tissue cultures were made from the cerebral hemispheres. These gave good growth of cells which were active and in good condition 187 days after the death of the fetus. They were then fixed and stained. Time lapse pictures were taken of some of the cells. There was little change in shape during 3 hours, though the granules in the cell were very active. Photomicrographs taken at 1 to 2 day intervals showed slight changes in the shape and position of the cells.

The cells were not oligodendroglia, nor microglia. They did not move like astrocytes. They had every appearance of small neurons with branched processes. Their slow locomotion also pointed to their being neurons.

39. CYTOLOGICAL CHANGES IN THE LIVERS OF OBESE MICE SUBJECTED TO A PROLONGED FAST. J. Walter Wilson and Elizabeth H. Leduc, Brown University. (Lantern; 15 min.)

Cytological changes have been studied in the livers of mice maintained without food but with water *ad libitum* at 78–80°F.: (1) obese mice up to 28 days; (2) lean mice up to 10 days; (3) lean mice receiving lard *ad libitum*.

The livers of the starved obese mice and of the lean mice receiving lard are very fatty. They show some damage after 16 days. Some fat laden cells become extremely distended and the fat globules tend to become confluent. Eventually all cells show this change. Necrotic areas result from their breakdown. The livers of starved lean mice that contain no fat show no such damage.

The cytoplasmic basophilia, indicative of "labile liver proteins," disappears early in starving lean mice, but in starving obese and lard-fed lean mice it may persist for 15-18 days. During starvation, body proteins are used rapidly as a source of calories in the lean mice, but in the other groups fat is utilized and cell proteins are drawn upon sparingly. As these proteins are used to the point of impairment, the liver cells are still loaded with fat. The cells then become distended. This may indicate a failure of fat metabolism and a loss of the cytoplasm which leads to cell destruction.

At the critical period the mitochondria become vesiculated and develop bizarre forms. This seems to be associated with the utilization of the essential cell proteins, possibly here involving the breakdown of lipo-protein complexes, and to contribute to the swelling of the cells.

40. SOME CONCLUSIONS REACHED FROM MICRODISSECTION STUDIES OF THE DIVIDING NEUROBLAST OF THE GRASSHOPPER EMBRYO. J. Gordon Carlson, Biology Division, Oak Ridge National Laboratory and Department of Zoology and Entomology, University of Tennessee. (15 min.)

Investigation with a microneedle of the internal structure of the mitotically active neuroblast growing in artificial culture medium leads to the following main conclusions: (1) The mitotic spindle is a semisolid body situated in a fluid cytoplasm. It appears to be lightly attached to the cell membrane at the poles, possibly by fine fibers connecting each pole to the adjacent region of the membrane. (2) During prometaphase, metaphase, and early anaphase, the chromosomes are securely attached to the spindle at their centromeres; distally they lie free in the cytoplasm. (3) As anaphase progresses the spindle appears to undergo liquefaction between the two groups of separating daughter chromosomes. The material of the interzonal regions is entirely fluid except for fine fibers connecting the distal ends of sister chromosomes. (3) Anaphase elongation of the spindle is an active process that is independent of any fibers connecting the spindle poles to the cell membrane. (5) Anaphase separation of chromatids is not simultaneous for the whole length of the chromosome; it begins proximally and gradually proceeds to the distal ends of the chromosomes. (6) Polarization of the neuroblast, which is manifest in unequal division, is independent of the spindle and chromosomes; it is apparently determined by the cell cytoplasm, the plasma membrane, the pressure of surrounding cells, or by a combination of these. (7) Formation of the cleavage furrow is independent of the spindle and chromosomes; its position, however, is, to a limited extent, determined by the position of the spindle and chromosomes.

Protozoology and Parasitology (Section D)

41. AN ABNORMAL TYPE OF DEVELOPMENT IN THE LIFE CYCLE OF TOKOPHYRIA INFUSIONUM. Maria A. Rudzinska, Washington Square College of Arts and Science, New York University, New York. (Introduced by Robert Chambers.) (Lantern; 10 min.)

An abnormality was observed in 6 unusually large individuals, each containing one very large brood pouch within which was a fully developed young Tokophrya possessing tentacles. Four individuals were starved and the young outlived the mother. Two individuals were fed and the mothers underwent encystment. In all there was an increase in size of the brood pouch and the contained young Tokophrya also increased in size. In the mothers a large number of contractile vacuoles was noted and their very rapid emptying. In the same culture were found individuals not in a stage of reproduction but with very large non-contractile vacuoles.

The embryos did not leave the brood pouch at the proper stage of development. An explanation proposed for this is that these individuals already possessed a large vacuole and that the brood pouch merged with the vacuole. The ciliated embryo thus completed its development in an abnormally large brood pouch. In the unfed mother Tokophrya the rapid disintegration of the body is possibly due not only to exhaustion from starvation but also because it was being sucked out by the young Tokophrya. An additional possibility is that waste products of the enclosed young Tokophrya exerted an adverse effect on the mother.

42. SURVIVAL OF ASTASIA LONGA FOLLOWING X-IRRADIATION. Henry W. Schoenborn, University of Georgia and Oak Ridge National Laboratory. (10 min.)

Pure cultures of *A. longa*, a colorless protozoan flagellate, have been subjected to 250 kv., 15 ma. X rays from a tube with an inherent filtration of 3 mm Al. The H. V. L. was 0.4 mm Cu. The intensity was approximately 1500 r/min., while total dosages ranged from 5,000 to 40,000 r. After irradiation single cells were aseptically isolated into tubes of complete medium. These tubes were incubated at 27°C. for 30-40 days, after which they were examined macroscopically for the presence of protozoan populations. Approximately 2,000 single cell isolations have been made at the 6 dosages employed. Lethality was not marked at 5,000 and 10,000 r, but became increasingly greater at higher dosages. When log survival was plotted against dosage, a multiple-hit type survival curve was obtained with the steep portion of the curve lying between 20,000 and 40,000 r.

43. EFFECT OF VISIBLE LIGHT ON GROWTH OF TETRAHYMENA GELEII. Austin Phelps, University of Texas. (Lantern; 10 min.)

A major factor in the inhibition of growth of *T. geleii* is found in the effect of light on the medium. Proteose peptone solutions which have been exposed to bright light will no longer support growth of the organisms even in the dark. Addition of a number of known vitamins to irradiated medium does not restore

its ability to support growth. If mixed bacterial cultures are grown in irradiated medium, it will then support growth. Addition of a small amount of proteose peptone will restore the growth value of irradiated medium. Strong light in the visible range destroys a growth promoting substance which is essential for *Tetrahymena*.

44. THE ROLE OF THIAMINE IN THE METABOLISM OF *TETRAHYMENA GELEII*.¹ Irving A. Tittler, Brooklyn College. (15 min.)

In thiamine-deficient medium with glucose, *Tetrahymena* causes an accumulation of pyruvic acid which results in an abnormally early decline of the cultures. Each experiment consisted of control and experimental series. To the latter the following concentrations of thiamine hydrochloride were added to the standard culture medium employed: 3×10^{-11} moles, 3×10^{-10} moles, 3×10^{-9} moles and 3×10^{-8} moles per liter. Determination of cell numbers per unit volume of culture medium, nitrogen content of the cells and pH were made from daily samples.

In these experiments, growth during the first 6 days of the control and experimental series, coincides very closely. By the 7th day a rapid drop in pH occurs in the control cultures and those containing 3×10^{-11} and 3×10^{-10} moles per liter of thiamine. Microscopic examination of these cultures fails to demonstrate the presence of living cells and chemical analysis reveals that an accumulation of pyruvic acid has occurred at this time. A lesser decline is found in cultures containing 3×10^{-9} moles per liter of thiamine. There is no such drop in pH and cell number in the cultures containing 3×10^{-8} moles per liter of thiamine nor is pyruvic acid present. Under the conditions of these experiments, the optimum concentration necessary for *Tetrahymena* to oxidize pyruvic acid, is about 3×10^{-8} moles per liter of thiamine. Concentrations greater than this are no more effective.

¹ Supported by a grant from the American Cancer Society upon recommendation of the Committee on Growth, National Research Council.

45. TEMPERATURE AND POPULATION LONGEVITY IN *TETRAHYMENA GELEII*. E. G. Stanley Baker and Sr. Jeanne d'Arc Schleichter, The Catholic University of America. (Lantern; 10 min.)

Populations of *Tetrahymena geleii*, strain E, were grown through the logarithmic phase at various controlled temperatures. A portion of each series was then transferred to a different temperature and population density and longevity in each series studied. Logarithmic phase temperature did not significantly affect the subsequent history of the population, but temperature during the total life of the population was important. In general, high temperatures favored higher population density and shorter population longevity.

46. THE EFFECT OF ALLYL ISOTHIOCYANATE UPON OXYGEN CONSUMPTION AND CARBON DIOXIDE OUTPUT OF SPIROSTOMUM AMBIGUUM. Harold E. Finley and Kenneth A. Tracey, Howard University. (5 min.)

Several methods for measuring the rate of respiration in protozoans have been employed by various investigators. Essentially the methods may be divided into two classes, (1) measurement of respiration in a group of organisms; (2) measurement of respiration in a single organism.

In this study a modification of the capillary method for single organisms was used. By means of finely drawn pipettes 0.5 NaOH was placed on each side of a specimen, confined in a capillary tube. The tube bore was 1 mm. Care was taken to prevent contact between the NaOH and the small drop of culture fluid which contained the Spirostomum. As oxygen was consumed the distance between the NaOH interfaces shortened. The quantity of oxygen consumed could be measured, in terms of the difference between the distance of the NaOH menisci at the start and at a given period of time later.

The same principle was employed in an indirect method of measuring the carbon dioxide output, with the NaOH drops being replaced by drops of olive oil.

Employing the above methods the mean oxygen consumption taken from the measurements of 100 different Spirostoma was 2.8093×10^{-3} cu mm per hour; the mean CO₂ output was 1.2573×10^{-3} cu mm/hr. These results seem to compare favorably with those of Specht (1934).

After exposing specimens to the fumes of a.i. for 10 seconds the O₂ consumption and CO₂ output was measured as described above. The following results were computed from the mean of 50 different specimens: O₂ consumption, 2.523×10^{-3} cu mm/hr.; CO₂ output, 1.036×10^{-3} cu mm/hr.

These results suggest that a change in the rate of O₂ consumption and CO₂ output accompanies the alterations in the cellular structure which occur when *Spirostomum ambiguum* is exposed to the fumes of a.i.

47. ENCYSTMENT STUDIES ON SPIROSTOMUM AMBIGUUM. Harold E. Finley and Henry A. Wise, Howard University. (Lantern; 5 min.)

Protective cyst formation can be induced in some genera of protozoa by subjecting the organisms to adverse environmental conditions. In this study *Spirostomum ambiguum* was subjected to the known encystment inducing factors.

Specimens were subjected to the following conditions: (1) Starvation by reducing the food content of the medium through repeated washings. (2) Crowding by concentrating large numbers in a small volume of medium. (3) Temperatures ranging from 38°C. to -4°C. (4) Dessication by exposing their culture medium to CaCl₂ and agar. (5) Changing the salt concentration by adding Knop's solution to cultures. (6) Decreasing the oxygen by sealing organisms in suitable vessels for prolonged confinement.

Atypical and spherical cyst-like forms or pseudocysts were produced but such forms failed to conform with the generally accepted criteria for cysts. Pseudo-encystment is regarded as a stage in the disintegration of the organism. True cyst formation was not induced.

Data indicate that hunger, dessication and reduction of oxygen, in the order given, are important factors in inducing pseudoencystment. Crowding, low temperatures, pH, and accumulated metabolic products appear to be negligible factors so far as they pertain to the formation of protective cysts by *Spirostomum*.

48. AN ANALYSIS OF THE EFFECTS OF A SACCULINID ON THE EXTERNAL MORPHOLOGY OF *CALLINECTES SAPIDUS* RATHBUN. Edward G. Reinhard, The Catholic University of America. (Lantern; 15 min.)

The rhizocephalid, *Loxothylacus texanus* Boschma, which infests the blue crab (*Callinectes sapidus* Rathbun) along the coast of Texas, produces striking effects on male hosts which tend to make them resemble females. The changes involve broadening of the abdomen, redivision of fused abdominal segments, pleopod alterations, and modifications of the dorsal abdominal musculature. Parasitized immature females assume the broad abdomen of the adult female and their pleopods are also altered, but not in the direction of maleness.

The parasitized males clearly demonstrate "parasitic intersexuality" which may be interpreted as resulting from a disturbance of the balance between male and female sex-formative substances brought about by the action of the sacculinid. The same type of parasitic activity, operating on a female host, should likewise increase the relative amount of female substance. Therefore immature females, instead of becoming intersexual are hyper-feminized and develop precociously the adult type of abdomen. The female pleopods, however, since they constitute a special type of secondary sex character concerned with the care of the developing young, are presumed to be independent of the sex substances but dependent upon the normal maturation of the ovary. Suppression of ovarian growth due to the parasite would interfere with their proper development.

These two-fold agencies, an upset in the normal relationship between the sex substances and the loss of ovarian control over the differentiation of the pleopods, together give a plausible explanation of the external effects of sacculinization, particularly in the case of *Callinectes* parasitized by *Loxothylacus*.

49. THE ABILITY OF THE AVIAN MALARIA PARASITE, *PLASMODIUM LOPHURAE*, TO INFECT ERYTHROCYTES OF DISTANTLY RELATED SPECIES. R. Barclay McGhee, Rockefeller Institute, Princeton, N. J. (Introduced by N. R. Stoll.) (Lantern; 10 min.)

Plasmodium lophurae, an avian malaria parasite, is able to infect the erythrocytes of man and the white mouse when such cells are present in the blood streams of infected chick embryos. Twice washed erythrocytes of 15 species of animals representing the classes mammalia, amphibia, and reptilia, were injected intravenously into infected avian embryos. Infections with *P. lophurae* were demonstrated in the cells of man, mouse, rabbit, and pig. The erythrocytes of these 4 animals are similar in their sodium-potassium balance. The individuality of various mammalian erythrocytes was indicated by their degree of susceptibility to the destructive forces of the embryo. Infections were not wholly dependent on the longevity of the introduced cell, since rabbit cells, which were able to

survive for only short periods of time, were susceptible, while bovine erythrocytes, with a long period of survival, were not infected. This selective infection indicates the role of the erythrocytes in natural resistance to malaria parasites.

50. FUNGI ASSOCIATED WITH TUMOR TISSUES. Irene Corey Diller, Institute for Cancer Research, Philadelphia, Pa. (Lantern; 15 min.)

The microscopic study of a variety of tumors (several transplantable tumors of the mouse; tumors induced in "A" strain and C3H mice by means of subcutaneous injections of methylcholanthrene; spontaneous mammary carcinomas of mice, human mammary carcinoma, positively diagnosed Hodgkin's nodes, etc.) by means of cytological techniques not ordinarily applied to tumor tissue, has led to the observation that there are present in these tumors spores and other fungal elements which in ordinary histological preparations appear to be integral tumor cells or are mistaken for lymphocytes and other blood cells. The organisms were readily isolated on Sabouraud's agar. Organisms subcultured for several transfers on agar, when re-injected into mice, produced inflammatory swellings which were sometimes resorbed and at other times were replaced by firm lumps of tissue. Cultures freshly isolated from tumors were highly pathogenic, causing death of all mice injected either intraperitoneally or intramuscularly within 4 to 5 days. Mycelial forms of the fungi were readily isolated from transplanted, induced and spontaneous tumors of the mouse and all types of human tumor tested. Preliminary indications are that the fungi are specific for particular types of tumor tissue.

51. STUDIES ON THE EXTRACELLULAR DEVELOPMENT IN VITRO OF AN INTRACELLULAR PARASITE (AVIAN MALARIA). William Trager, Rockefeller Institute, Princeton, N. J. (Lantern; 10 min.)

Malaria parasites, like the viruses and rickettsiae, are normally obligate intracellular parasites. Their development outside of a host cell has not been previously observed, and it may be that they are intimately dependent on special materials and structures of the host cell. It has now been possible to obtain, during an incubation period of two days *in vitro*, a partial development of avian malaria parasites freed from their host cells. The parasites (*Plasmodium lophurae*) were obtained from infected ducklings. The parasitized blood was suspended in a nutrient medium containing a concentrated extract of duck erythrocytes and was hemolyzed by treatment with an anti-duck-erythrocyte rabbit serum and guinea pig complement. The resulting suspension contained many parasites entirely freed from their host red cells. Small amounts of suspension were added to flasks with the nutrient medium, which was then jelled by the addition of either agar or fresh duck plasma. A liquid phase of nutrient medium was added and replaced with fresh medium at intervals of 12 hours. In the best preparations cell-free parasites of normal appearance could be readily found after two days of incubation at 40°C. Not only did segmentation of such parasites occur with the development of merozoites, but in addition some of the clusters of merozoites could be

seen to have begun development into young trophozoites. Such survival and development did not occur if the medium was not jelled or if it did not contain a sufficient concentration of duck-erythrocyte extract.

52. FREQUENT BUT APPARENTLY HARMLESS OCCURRENCE OF SARCOSPORIDIOSIS IN HEALTHY BEEF. Hsi Wang, American Meat Institute Foundation, The University of Chicago. (15 min.)

During a recent investigation of histology of beef, samples of *Longissimus dorsi* were secured from two steers at random at the Chicago Stock Yards. They were fixed in Zenker-formol or Bouin's within 50 minutes after slaughter. Celloidin sections, 10 μ thick, were subsequently cut and stained with Hematoxylin-eosin-azure (Maximow). Muscles of both steers were found to be infected by Sarcocysts with an intensity of approximately 5 cysts per cubic centimeter of tissue. The same parasite was encountered again in the *Triceps brachii* of a third steer taken later, and also in *Longissimus dorsi* of many more cattle used in another research.

The cysts range from small ovoid bodies, 0.04 mm by 0.06 mm, to typical "Mieschner's tube" 0.3 mm in length with an average diameter about 0.03 mm. The younger cysts are thinly enveloped in a homogeneous membrane; only the oldest ones are encapsulated with a striated wall. Each cyst is contained within the boundary of a single muscle fiber and is heavily loaded with spores. The latter resemble bananas in shape, having one end slightly more pointed than the other and attaining a fairly uniform size 3 by 10 μ . The grouping of spores into definitive lobules occurs only in older cysts. Typical sporoblasts (spore mother-cells) are present in some cysts. No sign of pathogenicity attributable to the parasite was observed. A comparison on the basis of morphology between the present parasite and Sarcosporidiosis in other animals reveals that it is more closely related to *S. tenella* (in sheep), *S. muris* (in mouse), and *S. hominis* (in man) than to those infecting ox or buffalo.

53. CRUSTACEAN PARASITES FROM BIMINI AND THE CAROLINA COAST. A. S. Pearse, Duke University. (Lantern; 15 min.)

Eighteen new species of barnacles copepods, and isopods were discovered in the following genera: Anuretes, Caligus (3), Lernanthropus (4), Hatschekia (4), Nemesis, Cybicola, Octolasmis (2) Leidyia, and Diplophryxus.

Animal Behavior and Sociobiology (Section A)

54. CERTAIN ASPECTS OF THE DEVELOPMENT AND BEHAVIOR OF THE WYOMING MOOSE — *ALCES AMERICANA* SHIRASI. R. H. Denniston, University of Wyoming. (Motion picture; 15 min.)

Development:

Weights, measurements, and pictorial record of a free-ranging calf moose were taken at regular intervals, and a record made of behavior changes during the first three months of life. This development was in contrast to the normal dependency of a calf on its mother.

Annual behavior cycle:

Spring: diet changes and the break-up of winter aggregations.

Summer: cow-calf and male-male groups.

Fall: male joining the cow-calf group; courtship and breeding behavior.

Winter: large mixed aggregations re-form; privations, parasitism increase; moose-human contacts are accentuated.

55. PATTERNS OF COOPERATIVE BEHAVIOR IN A HERD OF 14 GIANT TORTOISES AT THE BRONX ZOO. L. T. Evans and J. Quaranta, New York Zoological Society. (Motion picture, lantern; 8 min.)

The 6 *Testudo vicina* specimens were brought from the Galapagos Islands in 1928 (average weight 18 lbs.). Today they average 274 lbs.; the three *T. vandenburghi* average 222 lbs.; the two *T. ephippium* average 96 lbs.; the two *T. elephantina* (?) average 175 lbs.; the single *T. nigrita* (?) weighs 92 lbs. (resident at the Zoo since 1904). These 14 have been in the same quarters since September 1946. Prior to the latter date the *T. vicinas* were not a part of this herd.

This relatively static reptile society was studied by recording contacts between any two individuals while feeding, resting indoors both night and morning, resting outdoors, and in the numerical order of exit and entrance from and to the house.

The *T. vicinas* were especially sociable, the other species to a lesser degree. The regularity of the same kind of pairings was surprising consistent. Contacts were both intra- and interspecific.

In the numerical order of both exit and entrance, the *T. vicinas* usually preceded the rest of the herd. This order correlated to some extent with the order of dominance.

Many individual differences in behavior were observed. One male habitually leaves the house first or among the first 4 but he just as regularly enters the house after most are in. Another male regularly waits until another tortoise enters or leaves, usually a particular female, then he seems to take his cue from her; he has the deepest toned roar and is heard almost daily but only during copulation.

56. THE ROLE OF THE DISTAL TIP OF THE GONOPODIUM DURING THE COPULATORY ACT OF THE VIVIPAROUS TELEOST, *PLATYPOECILUS MACULATUS*. Eugenie Clark, Lester R. Aronson and Myron Gordon, American Museum of Natural History and New York Zoological Society. (Lantern; 15 min.)

In the Poeciliidae, the highly specialized gonopodium of the male is often referred to as an intromittent organ. However, its action during copulation has never been satisfactorily analyzed.

Six mature, virgin males (raised in the absence of young or mature females) were tested in a series of 10 observations with virgin females. A total of 506 gonopodial jabs and 36 copulations were recorded. Five control males tested at the same time jabbed 494 times and copulated 27 times. The tips of the gonopodia of the experimental fish were then amputated. After a recovery period of one

month a second series of 10 observations resulted in 2345 jabs and no copulation whereas the unoperated controls jabbed 1186 times and copulated 57 times. Another set of 11 males with amputated gonopodial tips were paired continuously with virgin females for 55 days. The females were checked regularly for the presence of sperm (by the oviduct smear technique). No sperm were recovered. It is concluded that the minute tip of the gonopodium is an essential structure for copulation and probably functions as a holdfast organ. The increase in sex activity after copulatory experience and the increase in jabbing frequency when copulation is prevented will be discussed along with the general sexual behavior and mechanism of insemination in this species.

57. THE INFLUENCE OF SOCIAL DOMINATION ON RECEPTIVITY IN THE DOMESTIC FOWL. A. M. Guhl, Kansas State College. (Lantern; 10 min.)

Various observations on a number of vertebrates have indicated that high social rank in a group is associated with precedence in sexual activity, especially among males. In flocks of chickens the most dominant cocks are most successful in mating and sire the most offspring. High rank among hens appears to interfere with mating. The passive dominance of cocks over hens facilitates mating. These results are of interest as they indicate an influence of psychological state (habit of dominating) upon reproductive behavior. There are some implications that selection for aggressiveness might occur in these flocks.

An effort was made to determine experimentally the influence of domination upon receptivity. These flocks composed of 28 to 40 females and from three to 5 males were used. The cocks were placed with the hens for brief, daily, observational periods. At the approximate peak of the laying cycle the flocks were divided into subflocks containing the hens of the top, middle, and bottom thirds of the peck-order. In this manner relative domination was reduced for the high ranking hens. The males were rotated between the pens of the subflocks. The rate at which the hens crouched for the males was used as an indication of relative receptivity. Over 10,000 crouches were recorded.

In all three flocks the hens composing the top third of the social order crouched least often, whereas, in subflocks the same hens crouched more than, or as much as, the hens from the other two levels of the peck-order.

58. HUNTING AND HERDING BEHAVIOR IN DOGS. Benson E. Ginsburg and Mildred J. Zamis, The University of Chicago and Roscoe B. Jackson Memorial Laboratory. (10 min.)

Ninety-five dogs belonging to 13 breeds and three crossbreeds were introduced singly and in groups into an acre field containing a herd of goats. Most of the dogs were reared under controlled conditions and could be certified as having had no experience with animals other than their mother and littermates. Of those whose history was incompletely controlled, none showed any deviations of behavior when compared with others of the same age and breed.

Trials were given daily for 5 days. Most dogs reacted aggressively by barking, chasing, stalking or direct attack. Some ignored the goats, and others avoided them. Most of the breeds studied could be rated on a scale of aggressiveness using these criteria.

Two cases of spontaneous "herding" behavior were noted: One in a Great Pyrenees male, the other in a Springer Spaniel female.

Basenjis never attacked when introduced singly, but attacked regularly in a pack. No other breed tested showed a comparable group effect.

In 6 instances, dogs of various breeds reacted "neurotically." They avoided the stress of reacting to the goats by becoming occupied with something else whenever the latter approached, i.e., excessive defecation and urination became substitutes for a positive or negative reaction to an approaching herd of goats.

59. THE FORMATION OF CONDITIONED AVOIDANCE RESPONSES IN YOUNG PUPPIES.

John L. Fuller, Clarice Easler and Edwin Banks, Rosecoe B. Jackson Memorial Laboratory. (Lantern; 15 min.)

Although the dog has been a classical subject for studies of conditioned reflexes, there is little information on the development of such reflexes in puppies. Thirty new-born puppies have been tested for conditioned avoidance responses to electric shock applied to the forelegs, using sound, light, odor and contact as conditioned stimuli. Receptors for odor and contact appear to function at birth, for light at about 16 days, and for sound at about 19 days. However, the age at which stable CR's developed to sound, light and odorous stimuli was from 18 to 21 days in all cases. Control puppies, not subjected to the conditioning procedure until 20 days of age, form CR's readily when training is commenced, and may be equal by the second day of training, to puppies trained since birth. CR's to contact were not produced by our technique. The time of appearance of conditionability corresponds to the beginning of the "critical period" of social development postulated by Scott, and to the changes in reflex patterns described by Bahrs. The evidence suggests important maturational changes in the central nervous system of dogs during the latter half of the third post-natal week.

60. INFLUENCE OF SPACE AND TIME ON THE SOCIAL BEHAVIOR OF THE RAT. John B. Calhoun, Johns Hopkins University. (15 min.)

The social relationships in a mature colony of Norway rats (*Rattus norvegicus*) after 28 months of population growth through 6 generations reflected the original pattern established during the first two generations. Some of these original relationships were established by chance, whereas others resulted from the interaction of the physical environment with the dissimilarities in behavior and physique of the different rats. Topics to be discussed include: (1) barriers and distance of harborage from the food source; (2) origin of weight differentials; (3) social continuity; (4) aggregations and hierarchies; and (5) mechanisms of self-limitation of population growth.

61. RADIOISOTOPES AS TOOLS FOR THE STUDY OF ANIMALS UNDER NATURAL CONDITIONS. Donald R. Griffin, Cornell University. (Lantern; 8 min.)

In many investigations of animal behavior it is most helpful to detect or record the movements of individuals with a minimum of artificial restraints or disturbance to their immediate environment. In some special cases where direct observation is difficult and where the spatial extent of the areas of observation is small, radioactive tagging of the animals is both feasible and effective. As an example, an instrument used in homing experiments with wild birds will be described, together with sample records showing the exact time at which the bird returned to its nest after transportation to a distance. This instrument could "watch" a bird's nest for 8 days without attention; and it proved itself practicable under field conditions in the Arctic. The calculation of effective and safe dosage and the choice of suitable radioactive materials will be discussed with a view to the application of the method to a variety of problems involving the behavior of animals under natural conditions.

62. BIRD ACTIVITY IN THE CONTINUOUS DAYLIGHT OF ARCTIC SUMMER. Martin Karplus, Harvard University. (Introduced by D. R. Griffin.) (Lantern; 7 min.)

The activity cycles of three nesting species of birds were studied in the continuous daylight of Arctic summer at Umiat, Alaska ($69^{\circ} 23' N.$ Lat.). Their sleeping rhythm was apparently governed by light intensity and the brooding rhythm by temperature. The active period of the adults was observed to extend over 21 hours per day. As a consequence of the increased number of feedings received each day by one brood of young robins (*Turdus migratorius*), their growth rate rose and their nestling period was significantly shortened (8.8 days in comparison with an average of 13.2 days in the northern United States). This is the first direct evidence for the theory that extensive migrations to the Arctic, despite their hazards for adult birds, have a survival value because of the more rapid growth of the young which is made possible by the continuous daylight.

63. RESPONSE LATENCY AND HABIT STRENGTH IN RELATIONSHIP TO SPONTANEOUS FIGHTING IN C57 BLACK MICE. Emil Fredericksen, Roscoe B. Jackson Memorial Laboratory. (Introduced by J. P. Scott.) (Lantern; 7 min.)

The present study is part of an investigation of the relationship between competitive and cooperative behavior in a variety of inbred mouse strains. Twenty male C57 Black mice, subline 10, were trained to fight under laboratory conditions with a high degree of environmental constancy. These animals fought spontaneously, in the absence of any physiologically significant traumata. It was found that the time at which a fight ensued for a given pair of mice decreased steadily, becoming asymptotic after the 4th trial. A decrease in response latency has in recent years been used as one of the measures of habit strength. The experimental results suggest the importance of habit formation in the development of social behavior.

64. ANALYSIS OF INDIVIDUAL DIFFERENCES IN PATTERNS OF CONFLICT BEHAVIOR IN DOGS. J. P. Scott and Margaret Charles, Roscoe B. Jackson Memorial Laboratory. (8 min.)

Consistent differences in behavior traits may hypothetically be produced by variability of (1) underlying physiological processes affecting activity, and (2) strongly fixed habits resulting from experience. The former is more likely than the latter to be importantly influenced by genetic factors. Results of these differences may be measured in terms of (a) ease of arousal and strength of internal (emotional and physiological) reactions, and (b) ease of arousal, choice, and modification of external behavior patterns. Examples of applying this sort of analysis to measurements of conflict behavior in dogs are discussed.

65. A DISCRIMINATION STUDY WITH THE AFRICAN ELEPHANT. Joseph A. Murnin and Dieter Bueckhardt, New York Zoological Society. (Introduced by T. C. Schneirla.) (Motion picture; 15 min.)

This study was conducted during the summer of 1949 at the New York Zoological Park.

The purpose of the study was as follows:

1. To develop adequate instrumentation and an experimental procedure in order to make possible discrimination studies with the elephant.
2. To determine the African elephant's sensitivity threshold to grey.

In this particular experiment one subject was used — a female African elephant 24 years of age.

The subject was first conditioned to the experimental situation with olfaction being controlled. The experiment proper consisted of 5 graduated intensities of grey, i.e., black was held constant, and white was varied. On each step the white intensity was increased toward black.

The entire experiment, including conditioning, required 2,183 trials. The point of J.N.D. was determined and this point was bracketed.

At present an experiment is being conducted to determine this particular elephant's ability to differentiate geometric form.

66. THE COLOR DISCRIMINATION OF *TESTUDO VICINA*. John V. Quaranta, New York Zoological Society. (Introduced by C. C. Little.) (Motion picture; 8 min.)

The preference of *T. vicina* for Orange was demonstrated by the following simple experiment. In a series of 10 trials, pairings of orange carrots (natural coloration) and carrots which has been vegetable-dyed a blue-green were presented to a specimen designated as R =. The animal consistently chose the natural carrot. In other series, blue-green carrots were presented in conjunction with orange color papers (abstract color). The abstract Orange was consistently chosen in preference to the blue-green carrots despite the fact that a pungent odor of carrot still remained in the dyed vegetables. In still another series, pairings of blue-green carrots were virtually neglected.

In a previous study, two animals designated as Experimental (R=) and Control (RO), were conditioned to discriminate among pairings of Green, Blue, and Orange Color papers of the same intensity. Both animals were then tested more exactly for Blue-Green discrimination. R= was required to respond positively to Blue; RO, positively to Green. Nine pairings of Blue and Green Color papers of different intensities and different saturations were used. Successes were significantly above chance. Finally, the experimental animal was tested for his ability to choose between a blue series and a series of 20 greys. The control animal was similarly tested for his ability to distinguish between a green series and 20 greys. Results were significantly above chance. Attempts are now under way to use filtered light.

Presumably, *T. vicina* is capable of blue-green discrimination.

67. A STUDY OF DOMINANCE ORDER IN A HERD OF CAPTIVE GIANT TORTOISES (TESTUDINIDAE) RESIDENT AT THE BRONX ZOO. John V. Quaranta and L. T. Evans, New York Zoological Society. (Introduced by C. C. Little.) (Motion picture; 7 min.)

In the present investigation, a herd of 14 giant tortoises was observed over a period of 6 weeks for the twofold purpose of detecting particular dominance clues and of establishing whatever dominance pattern might exist. Five species of Testudinidae were represented: *T. nigrita* (one); *T. ephippium* (two); *T. elephantina* (three); *T. vicina* (six); *T. vandenburghi* (two).

Preliminary observation and analysis by three competent observers indicated that if dominance techniques were to be detected, they would certainly occur in the two situations which are most crucial to the organism from the point of view of survival and well-being, i.e., the feeding situation (hunger drive) and the mating situation (sex drive). Fourteen criteria were tentatively adopted to determine provisionally the pattern of dominance.

Since it was impossible to observe the whole herd at any one time, a method of paired comparisons was developed which utilized the criteria tentatively adopted. Two independent observers recorded the frequencies of occurrence for the adopted criteria of dominance. For 30 separate two-hour observational periods over a 5-week duration, inter-rater reliability determined by Spearman's Rank Difference formula yielded a statistically significant coefficient: + 93. To obtain greater validity, the rank orders of dominance, established independently were averaged. Mean rank order was in turn correlated with several other variables. It was noted that Average Order of Dominance is significantly correlated with Order of Exit from the Shelter, but not with Order of Return to the Shelter.

General Physiology (Section B)

68. SOME EFFECTS OF ANTIBIOTICS ON DAPHNIA. Bertil G. Anderson, Department of Biology, West Virginia University and Franz Theodore Stone Laboratory, The Ohio State University. (15 min.)

Young *Daphnia magna*, less than 12 hours old, were exposed to various concentrations of bacitracin, clavacin, hemipyocyanine, penicillin, streptomycin, streptothricin, subtilin, tyrocidine, and tyrothricin dissolved in Lake Erie water. The

experimental animals were observed at definite intervals over a period of 100 hours and the number of survivors noted. Concentration-immobilization time curves were constructed.

All of the antibiotics studied were toxic to *Daphnia magna*. The differences in the concentration-immobilization curves indicate that no two of the antibiotics were effective in the same fashion. The most toxic antibiotic preparation was streptothricin which killed half the daphnids in 100 hours at a concentration of 87 mg per liter.

The results will be discussed in relation to the observations of others on the growth of individual daphnids and on the growth of daphnid populations. Antibiotics should be taken into account whenever studies of the causes of fluctuations in natural populations are undertaken.

69. FURTHER STUDIES ON GROWTH FACTORS FOR CARNIVOROUS CILIATES. Daniel M. Lilly, Anthony Pignataro and Kenneth Heilshorn, St. John's University, Brooklyn, New York. (Lantern; 15 min.)

A supplementary growth factor has been found necessary for the continued growth of certain hypotrichous ciliates feeding on smaller protozoa under bacteria-free conditions. For cultures of *Stylonychia pustulata* and *Pleurotricha lanceolata* feeding on ciliates of the species *Tetrahymena geleii* the required growth factor was obtained from yeast and several plant materials. An active concentrate of this factor has now been prepared by adsorption on charcoal and elution with ammoniacal ethyl alcohol. Attempts at substituting known vitamins of the B complex were unsuccessful. Further studies of the supplementary growth factor by chromatographic methods are now in progress.

More recently another hypotrichous ciliate, *Euplotes patella*, has been established in bacteria-free culture by supplying flagellates of the species *Chilomonas paramecium* as food. In this case a supplementary factor from yeast was also required. Before satisfactory growth could be obtained, however, it was necessary to add to the medium two additional factors, one supplied by a liver extract and the other by an extract of certain species of bacteria. Thus *Euplotes*, although feeding on a type of protozoa quite different from that used as food for *Stylonychia*, nevertheless appeared to require the same or at least a similar supplementary growth factor. Greater complexity in the nutritional requirements of *Euplotes* was indicated by the necessity of the two additional factors.

70. UNUSUAL PROPERTIES OF THE SUCCINOXIDASE SYSTEM IN THE CECROPIA SILKWORM. Richard C. Sanborn and Carroll M. Williams,¹ Harvard University. (Lantern; 15 min.)

The Cecropia silkworm shows an unusual organization of its cytochrome system in that the absorption bands of cytochrome *c* (at 550 m μ) and of *b* (at 565 m μ) are replaced by a single broad band extending from 551 to 562 m μ . This unusual component, which may conveniently be termed *b_s*, is the principal cytochrome in the larva. Only in the intersegmental muscles and the heart are

bands of cytochromes *b* and *c* encountered. Cytochrome *b_x* occurs in highest concentration in the walls of the midgut. Here, the only other spectroscopically visible component is cytochrome *a* + *a₃*.

Studies of intact midguts and of washed homogenates of midguts have shown that *b_x* possesses several peculiar properties: (1) like *c*, its oxidation is dependent upon cytochrome oxidase; (2) like succinic dehydrogenase and cytochrome *b*, it is typically bound to subcellular particles; (3) like *c*, it is rapidly reduced by ascorbate; (4) like *b*, it is rapidly reduced by succinate; (5) like *b*, it remains reduced when treated with urethane aerobically; (6) like *c*, its absorption band disappears in the presence of malonate; but (7) unlike *c*, the band does not reappear after the addition of ascorbate; (8) its ability to oxidize succinate and ascorbate is inhibited to approximately the same degree by malonate.

These lines of evidence suggest that cytochrome *b_x* is a spectroscopically visible component which combines the functions of succinic dehydrogenase, cytochrome *b*, and cytochrome *c*.

¹This study was aided by the Lalor Foundation and the American Cancer Society.

71. AMINO ACID AND NUCLEIC ACID RELATIONSHIPS IN *DROSOPHILA*. Taylor Hinton, John Ellis and D. L. Theriault, Amherst College. (15 min.)

Drosophila melanogaster was raised in the absence of microorganisms both on a chemically defined medium and on an autoclaved yeast medium. Certain amino acids when added to the medium in larger quantities exerted an inhibitory effect on growth. The inhibitory effect could be completely overcome in certain cases, at a given temperature, by increased amounts of nucleic acid in the medium. The study attempts to show which components of nucleic acid are antagonistic to the inhibition and the existing relationships at different temperatures.

72. ELECTRICAL CONTROL OF AXIAL POLARITY IN A REGENERATING ANNELID. Gordon Marsh and H. W. Beams, State University of Iowa. (Lantern; 15 min.)

Regenerating 6-12 somite sections of the fresh water annelid, *Aulophorus furcatus*, imbedded in agar, were exposed to direct current 4-5 days. Aerated tap water of specific resistance 2000 ohm/cm flowed continuously through the chamber at $25 \pm 1^\circ\text{C}$.

Pieces oriented anterior end to cathode regenerated normally. Pieces oriented to the anode through exposure: (1) regenerated normally at current densities below 5 microamperes/mm², but more slowly than control pieces, which developed in three days; (2) showed progressive retardation of development of the tail from 5 to 9 $\mu\text{a./mm}^2$, with occasional (and possibly questionable) retardation of the head near the higher density; (3) bipolarity, with two complete heads, at 9-10 $\mu\text{a./mm}^2$; and (4) reversal of the normal axis at and above 11 $\mu\text{a./mm}^2$. Potential gradients in millivolts/millimeter were 20 times the current densities.

Mortality was high, without consistent relation to current density. At varying times a proportion of the pieces underwent degeneration at one or both ends, making retardation, especially of the head, quantitatively uncertain. Turning of

pieces to the cathode was frequent; at any constant density above $5 \mu\text{a./mm}^2$ retardation of the tail was roughly proportional to the fraction of the last three days spent oriented to the anode. No retardation of the head was observed if turning occurred by the 4th day. Head and tail determination and control thus appear independent to a degree and not the simple inverse of each other.

73. QUANTITATIVE ANTIBODY STUDIES IN IMMATURE AND ADULT CHICKENS. Stata Norton and Harold R. Wolfe, University of Wisconsin. (Lantern; 12 min.)

Quantitative antibody studies showed that 6 to 9 week old chickens injected with beef albumin produced much less precipitins than adult birds. The duration of the antibody in the blood appeared to be longer in older animals than younger ones. Indications were that young chickens produced antibodies of very high molecular weight in addition to antibodies of known molecular weight (180,000) for adult chickens. There was no correlation between antibody content and spleen weight at 8 days after the injection of the antigen. There was no significant difference in the antibody concentration in the serum of young males and females.

74. THE EFFECT OF SPLENECTOMY ON PRECIPITIN FORMATION IN CHICKENS. Harold R. Wolfe, Stata Norton, Eleanor Springer, Morris Goodman and C. A. Herrick, University of Wisconsin. (Lantern; 10 min.)

The spleen of the chicken is concerned with antibody formation. Quantitative preceipitin studies showed that splenectomized animals produced an average of 9.2 gamma N antibody per milliliter while unsplenectomized birds averaged 282 gamma following a single injection of beef albumin. Splenectomized chickens were also less sensitive to minute injections of beef serum. When injected with large quantities or when given reinjection series antibody production seemed but slightly impaired, if at all.

75. THE EFFECT OF ADRENALECTOMY ON THE OXYGEN CONSUMPTION OF TESTIS OF TESTOSTERONE-TREATED NORMAL RATS. Jorge Gonzalez Q. and Clifford A. Angerer, Department of Physiology, The Ohio State University, Columbus. (Lantern; 12 min.)

Young white rats (20-30 gm) were divided into 6 experimental groups: (1) normal rats, (2) adrenalectomized rats, two groups of normal rats which received intraperitoneal injections of (3) 1 mg (light dosage), or (4) 5 mg (heavy dosage) testosterone propionate (Perandren-Ciba)/kg/rat/3 times/week/6 week period, and two groups (5, 6) treated as in "3" and "4" but adrenalectomized at least 4 days prior to experimental use. Respiration of the testis was studied by the Warburg manometric technique. The tissue was prepared as follows: the right testicles from ca. 6 rats, weighing from 80-120 gm and starved from 18-21 hours prior to use, were deprived of their tunica, were minced, and were pooled. To 200 mg samples (by torsion balance) was added 0.5 cc Krebs- PO_4 -Ringer's solution. To each of 6 respiration flasks was added 0.5 cc of this Krebs-testicular

mixture to 2.0 cc Krebs' solution. This solution had been buffered previously to pH 7.4 with Na_2HPO_4 . Equal portions of the mixture were placed in individual vials and dried overnight at 105°C . in order to obtain the dry weights of samples of testicular tissues. The experiments were performed in an atmosphere of pure oxygen and at a temperature of 37.5°C . The results are expressed as $\text{Q}_{\text{O}_2} = -\text{mm}^3 \text{ O}_2 \text{ consumed/mg(dry wt.) testis/hr}$. The numerals appearing in parentheses denote the number of rats used and also the number of experiments performed; normal rats, -1.8 ± 0.18 (16); adrenalectomized rats, -3.1 ± 0.21 (25); normal rats treated with light dosage of testosterone, -4.3 ± 0.26 (18), and with heavy dosage, -2.7 ± 0.11 (18); adrenalectomized rats previously treated with light dosage, -2.1 ± 0.16 (20); and with heavy dosage, -2.2 ± 0.12 (19) of testosterone.

76. PHOTOTROPIC RESPONSES IN AMEIURUS EMBRYOS AND THE ASSOCIATED RETINAL MORPHOLOGY. Philip B. Armstrong, Syracuse University College of Medicine and Marine Biological Laboratory. (12 min.)

The development of swimming in *Ameiurus* (bull-head) goes through stages similar to those described for *Amblystoma* by Coghill. There is (1) a non-motile stage, (2) a flexure stage, and (3) the "S" reaction and subsequently when this is rapidly repeated (4) locomotion results. Early in this last stage there is a succession of changing responses to light. Initially the embryos show no discriminative responses to light. The rods and cones and retinal pigment are first differentiating. This is followed by a period in which the embryos are markedly light negative. Rods and cones are differentiated over a large part of the retina but the myoids as yet show no functional changes. Both rods and cones appear as if contracted; there are no positional changes in light and darkness. The retinal pigment is expanded; it shows no positional changes in light and darkness. Though expanded it does not completely mask the rods and cones. In the next stage the embryos show a reversal and become light positive. The rods and cones and the retinal pigment now show the typical responses to light and darkness. These experiments may throw some light on the role of the pigment in the functioning of the rods and cones and also on certain aspects of encephalization.

77. EFFECT OF ACID-BASE CHANGES ON VISUAL THRESHOLDS. Charles D. Hendley, The Ohio State University. (Introduced by I. Rothschild.) (Lantern; 10 min.)

The absolute visual threshold in humans was lowered by about 35% when respiratory alkalosis was produced by voluntary hyperventilation (alveolar pCO_2 about 20 mm Hg; arterial pH 7.6). The threshold was raised by approximately the same amount during respiratory acidosis produced by breathing 6.8% CO_2 in O_2 (alveolar pCO_2 about 57 mm; arterial pH 7.3). These results are in general agreement with those of Wald et al. (J. Gen. Physiol., 25, 891, '42). On the other hand, preliminary experiments in which metabolic acidosis and alkalosis were produced by the ingestion of ammonium chloride and sodium acetate showed

the visual threshold to be unaffected by these changes in blood pH. It is concluded that the respiratory effects on the visual threshold are not due simply to altered pH, but result from the changes in carbon dioxide tension.

78. FAT METABOLISM IN THE ARCTIC GROUND SQUIRREL. Charles G. Wilber, Saint Louis University. (15 min.)

Specimens of the Arctic ground squirrel, *Citellus barrowensis*, (a discussion of the confusion in nomenclature will be presented) were caught in live traps in various parts of the Alaskan Arctic. Two large trappings were made: one in July, the other in September 1948. Samples of blood, liver and kidney from the various specimens were analyzed for fatty acids, cholesterol, phospholipid. The results show that total fatty acids and cholesterol are high in the blood of the squirrels, as compared with ordinary laboratory animals, but that cholesterol is about the same as in the dog. Blood from July squirrels is statistically the same in lipid content as that from September animals.

On the other hand, there is almost three times more fatty acid and two times more phospholipids in the livers of July squirrels than in those of September squirrels. Cholesterol is the same in both. In the kidneys a similar picture of the lipids is obtained.

The results suggest that there is a rapid rate of fat metabolism in the Arctic squirrels during the short summer months, with a gradual decrease in lipid turnover as hibernation approaches.

79. BIOCHEMICAL CHANGES DURING THE EMBRYONIC DEVELOPMENT OF POPILLIA JAPONICA. Fred Rothstein, University College, New York University. (Introduced by H. W. Stunkard.) (15 min.)

Japanese beetle eggs, kept at 30°C., were subjected to biochemical analysis daily during the 8 day embryonic period. The freshly laid egg weighed 0.8 mgm and the maximum weight of 2.48 mgm was closely approximated on the third day of development. This increase in weight was due to absorption of water. Upon emergence there was a loss of 0.54 mgm, accounted for by shading of the chorion and the loss of water and carbohydrate. The water content varied from 0.42 mgm, in the newly-deposited egg, to 2.12 mgm on the 7th day and upon hatching the amount of water was reduced to 1.54 mgm. The total N content remained constant at 0.036 mgm throughout the embryonic period. Glycogen showed an irregular decrease from 0.021 mgm, in the freshly-laid egg, to 0.007 mgm, in the newly-hatched larva. There was a rapid fall during the first three days of development. Following this decrease a period of constancy persisted to the 5th day, after which a further decrease occurred until hatching. Glucose rose from 0.002 mgm on the initial day to a maximum of 0.007 mgm on the the third day. The 4-day egg showed a lower reading, 0.005 mgm glucose, followed by a very gradual increase to 0.006 mgm on the 7th day. The newly-emerged larva showed a lower value of 0.005 mgm. Glycogen appears to serve as the source of glucose, which supplies the energy for embryonic development. Determinations of free fat are in progress.

Endocrinology (Section C)

80. RELATIVE SENSITIVITY OF THE IMMATURE AND PREGNANT MOUSE FOR ASSAY OF UNFRACTIONATED PITUITARY GLANDS. A. J. Ladman, Roscoe B. Jackson Memorial Laboratory. (Introduced by Meredith N. Runner.) (15 min.)

It was early observed that gonadotrophic hormones both stimulated the reproductive systems of immature mice and produced ovulations in mature females. Although both end points have been used for assay of hormones of the pituitary gland the relative sensitivities of the two are unknown. Therefore comparative assays were made of unfractionated, dried pituitary glands of post-parturitional mice.

The first series, started on mice 16 days of age and 5.5 to 7.5 gm, was made by injecting males intraperitoneally and females subcutaneously twice daily for 4 days and autopsying on the morning of the 5th day. The second series, made on mice 15 to 19 days pregnant, gave results 18 hours after a single injection. Assays of 1/2, 1, 2 and 4 pituitary equivalents showed that at doses of 2 and 4 pituitaries the uteri gave 79% and 296% greater organ-body weight ratios than that of the controls, the ovaries responded with 19% and 37% increases and the seminal vesicles resulted in 63% and 112% responses respectively. Thus the minimal dose to produce a 100% response from the uterus and the seminal vesicle was 4 pituitary equivalents. Assays of 1/8, 1/4, 1/2, 1 and 2 pituitary equivalents found the critical, 50% ovulatory response at the dose of 1/2 pituitary (19 animals injected, 47% had tubal ova). Findings from the two methods of assay are interpreted to indicate that the pregnant mouse was approximately 8 times as sensitive as the immature mouse for assay of these pituitary glands.

81. ESTROGEN LEVELS IN THE NON-OVULATING RABBIT. Clara E. Hamilton, University of Georgia. (Lantern; 10 min.)

An investigation is being made to determine if there are cyclic alterations in the estrogen output of the non-ovulating rabbit, since, in this organism, there is always a continual series of ripening and degenerating follicles, and there are always present follicles capable of maturing and rupturing within 10 hours after an adequate stimulus. Studies of the vaginal smear (using the Shorr staining technic and procedure), histology of the reproductive tract, and blood estrogen levels are being made. The vaginal smear, thought to reflect estrogenic output, shows cyclic recurrence of peaks of relative abundance of certain distinct cell types at 4 to 6 day intervals. Location of the source of certain of these cell types has been made. The blood estrogen levels are being investigated by injecting whole rabbit blood into previously primed ovariectomized guinea pigs (Hartman-Littrell method); opening of the vaginal closure plate being a positive response. Apparently at the time cornified cells are relatively more abundant there is a higher blood estrogen level.

82. THE EFFECT OF DIETARY DEFICIENCIES AND ENDOCRINE ALTERATIONS ON THE APYRASE AND THE D-AMINO ACID OXIDASE OF RAT TISSUES. Samuel R. Tipton, Frances M. Colvin and Kurt Weiss, Department of Zoology and Entomology, The University of Tennessee. (Lantern; 10 min.)

Additional data have been obtained on the effect of vitamin B deficiency on the rat liver and kidney apyrase in conditions of thyroid and adrenal disturbance. Contrary to an earlier report the liver and kidney apyrase activity of rats maintained on casein-sucrose-vegetable oil with adequate supplements of vitamins is fully as high as that of rats on Rockland diet. In general the experimental procedures have had no significant effect on kidney apyrase or oxidase. The average liver apyrase value of 93.3μ g.P per millogram dry weight tissue per hour is raised significantly by the administration of thyroid powder. Neither the normal value nor the rise with thyroid is affected significantly by a total B deficiency or thiamin deficiency alone. In riboflavin deficiency the apyrase activity appears to be higher than in controls. This high value is not significantly increased by thyroid administration. After adrenalectomy liver apyrase is higher than that of controls; but on administration of 7-8 ml of lipoadrenal extract the activity level is markedly lowered.

Riboflavin deficiency results in a significant decrease in liver d-amino acid oxidase. After thyroxine the liver enzyme values of these deficient rats are not significantly different from controls on normal diet and normal thyroid levels. After adrenalectomy the oxidase activity is relatively unaffected rather than decreased as we reported earlier.

The data reported here indicate that riboflavin is involved in some manner with the regulation of activity of both liver apyrase and d-amino acid oxidase. The level of these enzymes in the tissue can be increased under the influence of thyroxine even in a condition of riboflavin deficiency.

83. SPECTROPHOTOMETRIC ANALYSIS OF FEATHER PIGMENTS FROM ADULT BROWN LEGHORN CAPONS AND THYROIDECTOMIZED ROOSTERS. Ben B. Blivaiss and Sherwin Zalman, Department of Physiology and Pharmacology, The Chicago Medical School. (15 min.)

Spectral analyses were made of filtered, alkaline hydrolysates of black breast and red-pigmented saddle feathers plucked from each of 5 capons, and red-pigmented breast and saddle of 5 thyroidectomized roosters. An extract of White Leghorn feathers was used as blank for determination of absorption spectra on the Beckman spectrophotometer to compensate for presence of unknown materials.

The pigments were insoluble in 95% ethanol, benzene, and acetone confirming that they are melanins rather than carotenoids.

In the 4 feather types studied at 20 m μ intervals from 220-1000 m μ , light absorption decreased with increasing wave-length. No well-defined absorption peaks were evident. Transmission was greater for red pigments than for black one. Angle of slope of optical density (T) curve for capon breast was greater than for the three red feather types which were similar.

Oxidation of black or red pigment solutions with hydrogen peroxide produced a light yellow to nearly colorless solution which had a decreased absorption and

an increased angle of slope of E curve compared to control of same dilution. Reduction of black or red pigment with ascorbic acid resulted in a dark brown or black solution which had an increased absorption and a decreased angle of slope of E curve compared to control. A well-defined peak was found at 500 $m\mu$.

Red pigments in capons and thyroidectomized roosters appear to be similar on the basis of spectrophotometric analysis. Effects of hydrogen peroxide and ascorbic acid suggest a similarity in chemical nature of black and red pigments.

84. EFFECT OF ADRENOCORTICOTROPHIN¹ ON THE ADRENAL GLAND OF THIOURACIL TREATED RATS. M. X. Zarrow and I. G. Zarrow, Purdue University. (Lantern; 10 min.)

It has been definitely established that atrophic changes take place in the adrenal glands of rats treated with thiouracil. This involution may be due to a direct action of the drug on the adrenal cortex or to an indirect action mediated by way of the pituitary gland. In order to investigate this point, female rats were given a solution of 0.1% thiouracil in their drinking water and kept on this treatment until the end of the experiment. After 12 weeks of thiouracil treatment the rats were injected with adrenocorticotrophin¹ (ACTH) twice daily for 6 days, killed on the 7th day and the adrenals removed and weighed. Suitable controls were maintained.

In three different experiments it was observed consistently that the adrenal gland of the thiouracil treated rat was as sensitive to ACTH as the adrenal gland of the normal rat. In one experiment ACTH caused an increase of 29.5% in the adrenal weight of animals receiving thiouracil and 32.3% increase in the adrenal weight of normal, untreated control animals. These results indicate that the adrenal gland of the thiouracil treated rat is capable of responding to ACTH and consequently it would seem that the involution of the adrenal cortex after thiouracil treatment is due to an indirect action of the drug.

¹ Obtained through the courtesy of Dr. John Mote, The Armour Laboratories, Chicago.

85. EFFECT OF ULTRASONIC VIBRATIONS ON THE MOLTING RESPONSE OF TRITURUS VIRIDESCENS. Hugh Clark, University of Connecticut. (Lantern; 10 min.)

Early experiments demonstrated that Triturus responds to ultrasonic vibrations by increasing the frequency of molting. Animals were exposed to vibrations of 350,000/second generated by a piezoelectric instrument at an energy level of approximately 50 watts acoustic. A subsequent series of 16 experiments divided among some 200 animals shows: (1) no significant difference in response to intensities between approximately 5 and 60 watts, providing longer exposures were used at lower energy levels; (2) no consistent alteration of response at higher energy levels due to variation of duration of exposure from 10 to 90 seconds; (3) exposure of head or tail to be slightly more effective in increasing the molting rate than exposure of the entire body; (4) no correlation between degree of response and season when salamanders were treated; (5) retention of the effect of treatment for periods as long as two months. The average interval between

molts in control animals was found to be 21.3 days. The increase in rate of molting of treated animals over that of controls is $2.3 \times$ (head), $2.4 \times$ (tail), and $1.9 \times$ (entire body). Attempts are in progress to ascertain whether the effect is on the skin, thyroid or pituitary, the most likely sites, according to the interpretation of the molting process by Professor Adams and her co-workers.

86. SOME FACTORS INFLUENCING THE CHOLESTEROL CONTENT OF THE INTERSTITIAL TISSUE OF THE IMMATURE RAT OVARY. Edward G. Rennels,¹ Harvard University. (Introduced by Alden B. Dawson.) (Lantern; 15 min.)

Schultz and Schiff reactive materials make their initial appearance locally in the interstitial tissue of the rat ovary at about the 10th to the 12th day of life. These materials gradually reach a maximum distribution about the 15th day when the entire interstitium is reactive. High concentrations persist until the first ovulation. In the intact immature animal single injections of PU or PMS will bring about the total mobilization of the cholesterol in the interstitium. Longer treatment with these gonadotropins induces vaginal changes indicative of estrogen secretion by the 15th day. The follicular thecae of such ovaries is non-reactive. If hypophysectomy is performed on the 26th day the interstitial cells retain their high concentration of cholesterol and plasmalogen. Both cholesterol and plasmalogen can be mobilized in hypophysectomized animals by injections of PU or hypophyseal LH, and both will accumulate again soon after the injections are suspended. Signs of estrogen secretion can be detected following the administration of these gonadotropins only when the injections are begun soon after the operation. Daily injections of estradiol initiated prior to the 10th day of life will completely prevent the normal accumulation of cholesterol and plasmalogen up to at least the 25th day. The ovaries of animals treated in this manner are characterized by (1) the absence of cholesterol and plasmalogen in the interstitium and (2) the absence of typical atretic follicular residues. Such atretic residues which are clearly of thecal origin are very conspicuous in the 25-day normal ovary.

¹ AEC Fellow in biology.

87. THE ANDROGENIC ACTIVITY OF OVARIAN TRANSPLANTS TO THE SEMINAL VESICLE OF THE CASTRATED ADULT MALE RAT. Seymour Katsh, Department of Biology, New York University, Washington Square East, New York. (Introduced by Harry A. Charipper.) (Lantern; 15 min.)

Ovaries obtained from varying aged virgin female and pregnant female rats when homotransplanted to a seminal vesicle of the adult castrated and castrated-adrenalectomized rat ameliorates the precipitous regressive changes in the reproductive accessories which are the usual sequelae of castration. This androgenic capacity is reflected in histologically and cytologically evident signs of stimulation as well as partial weight maintenance of the seminal vesicle-prostate complex in gonadectomized hosts. Implants of 15-day old testis as well as autografts of this material also serve to maintain the accessories, but transplants of control tissues did not alter the castrate condition.

Cholesterol, estrone and progesterone crystals introduced into the vesicle have no androgenic effects but the efficacy of testosterone crystals is well demonstrated since each vesicle receiving this steroid weighed more than twice that of the normal.

Microscopic examination of the serially sectioned grafts recovered at the end of 20 days shows most ovaries to be essentially normal in follicular and corpora lutea development. Correlation of ovarian histology with the degree of androgenicity appears to involve luteinization of the theca interna of the follicles although non-luteinized grafts also possess some stimulating capacity. The testicular implants assume the typical cryptorchid condition.

It appears therefore that ovarian grafts to the essential vesicle of the adult castrated rat continue to secrete sufficient amounts of androgen to maintain the accessories of the castrated adult male host and further that the ovary thus grafted maintain its histological and physiological differentiation and may be considered as essentially normal at the termination of the experiment.

88. CHANGES IN REPRODUCTIVE AND METABOLIC FUNCTIONS IN MICE GIVEN RADIO-TOXIC DOSAGES OF I^{131} . Aubrey Gorbman and Hilda Beim, Barnard College, Columbia University. (Lantern; 15 min.)

Mice of the C57, I, and CFW strains fed a Purina chow diet and given thyroid-lethal, or nearly thyroid-lethal (100 to 200 microcuries) quantities of carrier-free I^{131} , develop tumors of the pituitary gland in high incidence about 8 months after treatment. Interest attaches, therefore, to endocrine or non-endocrine disturbances which may play a role in development of such growths.

Young mice treated with I^{131} plateau earlier, and at a lower body weight, than untreated controls, but seem to grow and differentiate fairly well nevertheless. Oxygen consumption, after an initial fall, rises to nearly normal levels, and sometimes even exceeds control values. A gradual fall in oxygen consumption occurs in the one or two pre-mortem months.

In the ovary ovogenesis is stopped quickly in most instances and accumulation of luteoid tissue occurs. Testicular function appears quite normal histologically. Though rare, a few pregnancies occur up to as much as 5 months after I^{131} treatment. Especially in C57's increasingly irregular vaginal estrous cycles lead to long periods of cornified vaginal smears.

89. THE RELATION OF THE NUMBERS OF CORPORA LUTEA AND OF EMBRYOS IN WILD RATS. Octavia Hall and David E. Davis, University of Texas Medical Branch and Johns Hopkins University. (Lantern; 15 min.)

The ovaries of wild rats (*Rattus norvegicus*) were sectioned serially to determine the number of corpora for comparison with the number of embryos. The rats were captured between January 1, 1946 and March 3, 1947, in poultry warehouses in Baltimore, Maryland. The ovaries of 55 pregnant rats had 603 corpora and 513 embryos (1.17 corpora per embryo). However, 30 of the rats had more corpora than embryos, 17 had an equal number, and 8 (14.5%) had less corpora than embryos. The presence of an excess of embryos is explained in part by the appearance of polyovular follicles in at least 12% of the ovaries examined.

90. THE RELATION OF DAY LENGTH AND GONADAL REGRESSION TO POSTNUPTIAL MOLT IN THE WHITE-THROATED SPARROW AND OTHER FRINGILLIDS. Albert Wolfson, Northwestern University. (15 min.)

Juncos, White-throated Sparrows, White-crowned Sparrows, and other fringillids were subjected to various conditions of day length and observations made on the gonadal response and postnuptial molt. The results of a large series of experiments demonstrate conclusively that the postnuptial molt can be induced in late winter or early spring by subjecting birds to *constant* day lengths of either 24 hours, 20 hours, or 15½ hours beginning December 4. In each case the onset of the molt was correlated with the regression of the testes from maximal size.

When birds were subjected to 9 hours or 12 hours of constant day length beginning December 4 they failed to undergo a postnuptial molt. Even after 20 months there was no postnuptial molt. In the 9-hour group the testes failed to recrudescence, whereas in the 12-hour group they reached breeding condition but failed to regress completely.

In another series of experiments, birds were captured during the spring migration in May and subjected immediately to 9 hours of constant day length. The testes, which were about one-half the maximum size at the start of the experiment, began to regress but there was no postnuptial molt.

The results of these experiments and other observations demonstrate clearly that in these species decreasing day length and/or gonadal regression *per se* are not the causes of the postnuptial molt.

Cellular Physiology (Section D)

91. UTILIZATION OF ATMOSPHERIC NITROGEN BY THE ANIMAL MICROORGANISM, COLPIDIUM CAMPYLUM. Gerald R. Seaman, Creighton Medical School. (Introduced by John Frisch, S.J.) (Lantern; 15 min.)

A pure, sterile culture of the ciliate protozoan, *Colpidium campylum* was established in a medium consisting of 0.1 M sucrose in Hahnert's solution. Analyses indicated that the medium contained less than 1 gamma of Kjeldahl nitrogen, 5 gamma of nitrate, and 0.1 gamma of nitrite per liter. One-half milliliter of inoculum from a proteose-peptone culture was transferred into 10 ml of the sucrose medium. When maximum growth was reached, 0.5 ml of this culture was transferred to a second 10 ml of sucrose medium. This procedure was repeated through 10 transfers. At the 10th transfer, calculations indicate that less than 1.8×10^{-5} gamma of material per liter had been carried over from the original peptone medium. After 10 transfers in the sucrose medium, the size of the organisms decreases to approximately half the dimensions of the organisms grown in the peptone medium. However, the general morphology of the cell remains constant. At no time were disfigured organisms showing blebs found, as had been reported in the case of *Tetrahymena* when grown in sucrose solutions. The growth rate in the sucrose medium is very much lower than that obtained in peptone medium. Maximum populations reach a level of about only 16,000 organisms per milliliter. Manometric measurements show that starved cells from the peptone medium do not utilize sucrose. This ability is gradually

generated through successive transfers in the sucrose medium. The ability of *Colpidium* to grow in a medium devoid of nitrogen indicates that the organism is capable of fixing atmospheric nitrogen. Manometric measurements show that, in fact, the organisms do fix nitrogen from the atmosphere.

92. FOURTEEN-DAY RHYTHMS AND SURFACE RELATIONSHIPS OF LEUCOCYTES AND AMEBAS. A. A. Schaeffer and Carol Honegger, Temple University. Lantern; 15 min.)

Further work on rabbit leucocytes definitely establishes a 14-day rhythm in the left/right spiraling ratio. As reported earlier, a 14-day rhythm is also present in oyster leucocytes and marine abas (*Flabellula*) and is indicated also in man. The left/right ratios are more numerous at 1.40, 2.05 in amebas and at 2.00, 2.80 and 4.00 in oyster leucocytes, at 1.40, 1.75, and 1.95 in rabbit leucocytes and 1.35, 1.75, and 2.00 in human leucocytes. All leucocytes studied change direction more frequently at about 85 μ , 119 μ , and 170 μ , while the amebas change direction more frequently at 122 μ , 170 μ , and 243 μ . An X-ray mutation of the multinucleate ameba *Chaos chaos* Linn. stabilized at 0.7 the diameter of *Chaos chaos*. The other mutations, of the same surface relationship were unstable and in a few months changed back to the size of the permanent mutation. All these quantitative relationships appear to be based on the $\sqrt{2}$ and are therefore probably concerned with the surface of amebas and leucocytes. This extraordinary uniformity in the characteristics of ameboid cells raises questions concerning similar behavior in other less strongly ameboid cells such as fibroblasts and even nerve cells. The hypothesis is presented that the $\sqrt{2}$ relationship reflects a plasma membrane or ectoplasmic structure of elements (molecules) associated in n , $2n$, $4n$, etc., groups, predominantly, which form loose surface networks.

93. HISTORIES OF IMPLANTS OF FOREIGN SUBSTANCES IN FROG TADPOLES. Carl Caskey Speidel, University of Virginia.¹ (Lantern; 15 min.)

Implants of several kinds of foreign substances have been observed microscopically in frog tadpoles from day to day for periods of several months. Carmine powder and zinc oxide powder, implanted in the tadpole's tail, evoke cellular reactions quite like those previously described for tantalum powder. These include the early response of the leukocytes which quickly congregate in the implant vicinity; the picking up of the powder particles by leukocytes, fibroblasts, and lymphatic endothelial cells; the later elimination of much of the implant by cutaneous papilla formation and pinching off; and the slow migration of many of the powder-laden leukocytes by way of the lymph and blood vessels. Occasionally encapsulation of a sizable portion of an implant occurs. Some powder-laden macrophages may remain at the implant site for months.

Implants of silk or nylon strands (also cellophane fragments) induce reactions like those caused by tantalum wire. The strand is quickly walled off by leukocytes. Many of these unite to form a delicate flat syncytial layer about the implant. Sometimes a strand may change its position and in the process shed some of the encasing leukocytes.

Implants of soft paraffin are quickly invaded by leukocytes which soon become loaded with paraffin globules. Implants of hard paraffin are isolated by a leukocyte shell. Implants of tar fragments are similarly isolated. There is also some ingestion of very small tar granules.

Illustrative cine-photomicrographs have been obtained (cf. demonstration no. 157).

¹ Aided by a grant from the American Cancer Society (Committee on Growth).

94. EFFECT OF ULTRAVIOLET RADIATION ON THE RATE OF CELL DIVISION OF *ARBACIA* EGGS, AND THE ENHANCEMENT OF RECOVERY BY VISIBLE LIGHT. H. F. Blum, J. P. Price, J. C. Robinson, and G. M. Loos, National Cancer Institute, Department of Biology, Princeton University, and Marine Biological Laboratory, Woods Hole. (Lantern; 15 min.)

Appropriate dosage with ultraviolet radiation (wavelengths 2700A to 3130A) slows the rate of cell division in *Arbacia* eggs, without permanent damage. Recovery of normal rate is gradual and complete. Under comparable conditions the rate of recovery is about the same if the ultraviolet radiation is applied before fertilization, between fertilization and first cleavage, or between first and second cleavages. Application of ultraviolet within about 20 minutes prior to a given cleavage fails to delay that cleavage, but does delay subsequent ones. This tends to obscure the smooth character of the recovery process, which goes on whether cell division occurs or not.

Recovery is markedly accelerated by illumination with light from the blue-violet and near ultraviolet region of the spectrum (long wavelengths limit ~5000A), after dosage with ultraviolet. The white halves of centrifuged eggs exhibit the same recovery phenomenon. Such light does not enhance recovery if applied before dosage with ultraviolet, nor does it accelerate the rate of cell division of normal eggs. The effect seems essentially the same as that described by Kelner in fungi (Proc. Nat. Acad. Sci. 1949, 35, 73), and Dulbecco (Nature 1949, 163, 949) in bacteria; and possibly that found in *Fecus* by Whitaker (J. Gen. Physiol. 1942, 25, 391).

Similar results are obtained when X-rays are applied to the eggs in appropriate dosage, except that "visible" light does not enhance the recovery. This important exception indicates, however, a fundamental difference in the mechanism of action of ultraviolet radiation and of X-rays.

95. *ARBACIA* SENSITIVITY TO DIMETHYLATED DIOXYPURINES. Ralph Holt Cheney, Brooklyn College and the Marine Biological Laboratory, Woods Hole. (Lantern; 15 min.)

Theobromine and theophylline, the chemical agents employed, were prepared in the following molarities-in-sea-water—TbSW M/1000, M/600, M/400; TpSW M/1000, M/600, M/400, M/200, M/40. Ten developmental stages of *Arbacia punctulata* (fertilization membrane, 2-, 4-, 8-celled, late cleavage, blastula, gastrula, prism, and young pluteus) were transferred from normal sea water to

the appropriate concentration of TbSW or TpSW. This constituted a sensitivity series to ascertain the stages of development at which the physiological processes of the organism were affected by these dimethylated dioxypurines. Several hundred specimens were observed during each experiment during which control and experimental cultures (all developed from the eggs of one female and fertilized by the sperm from one male) were maintained in stender dishes surrounded by running sea water. Periodic examination and photomicrographic records were made at $100\times$ and $500\times$ (water immersion lens).

The sensitivity of *Arbacia* to the trimethylated dioxypurine, caffeine, has been shown previously by the author (Anat. Rec., 96, No. 4, Dec. 1946). Comparisons can be made. Retardation in time required for mature pluteus larval development under the influence of theobromine and theophylline has been demonstrated by the author (Bio. Bull., 97, No. 2, Oct. 1949). Equivalent molarity studies of theobromine and theophylline on each of the 10 stages presented currently indicate:

1. Cell division is retarded slightly during the first cleavage.
2. Blastula-gastrula-prism-young pluteus stages are retarded drastically.
3. Order of decreasing effectiveness of equivalent molarities is Tb, Tp.
4. Maximum solubility concentration (M/40) of Tp inhibits all further growth.

96. GRADUAL INHIBITION OF A POPULATION OF DIVIDING SEA-URCHIN EGGS BY HYPOXANTHINE AND THEOPHYLLINE. Ivor Cornman, George Washington University. (Introduced by A. F. Shull.) (7 min.)

A number of purines, including some folic acid antagonists, differ from most mitotic poisons in that the purines produce a graduated blocking of *Lytechinus* eggs. Hypoxanthine at 1000 and 2000 mg/L finally brings development to a half when some eggs are 32-celled, while others had already been stopped at 1, 2, 4, 8, and 16 cells. Halving the dose permits a 10- to 20-fold increase in the number of eggs that divide. It seems probable, then, that even the usually small variations among the eggs of one urchin are adequate to permit some of the eggs to divide several times more than others.

The effects of theophylline at 2000 mg/L resemble those of hypoxanthine. The block induced by 1000 mg/L is also spread over many cleavages and permits some eggs to continue through the blastula stage. This resembles adaptation, but retesting the "used" theophylline solution shows it has lost some of its potency. Also, eggs at the 4-cell stage are slightly more resistant than eggs just fertilized. A blocking dose added gradually between fertilization and third cleavage is no less effective than instantaneous administration of the whole dose between second and third cleavage. Accordingly, the continued development of a portion of the eggs in a blocking dose of theophylline appears to result from the combined effects of individual variation, inactivation of some of the drug, and the lower susceptibility of later developmental stages.

97. CONCERNING THE SIGNIFICANCE OF THE AFFINITY OF MOTOR END-PLATES FOR JANUS GREEN B. John H. Welsh and Sumner Zacks, Harvard University. (Lantern; 15 min.)

The portion of a motor end-plate that stains supra-vitally with Janus green B, following the method of Couteaux, may represent a concentration of acetylcholine-receptor substance and acetylcholine esterase. This view is based on the observation that Janus green B applied to certain tissues has an acetylcholine-like action, and that it also acts as an anti-cholinesterase. Motor end-plates of skeletal muscle of the lizard, *Anolis carolinensis*, stain blue-green with $1:10^5$ Janus green B. Soaking the muscle in a solution of d-tubocurarine before staining, in an attempt to block acetylcholine-receptor substance, does not appreciably alter the staining reaction. After pretreatment with $1:10^5$ di-isopropylfluorophosphate (DFP), however, the motor end-plates do not stain normally. Neostigmine interferes less than DFP with the staining reaction. Muscle of the heart of *Venus mercenaria* and amoebocytes of this same bivalve mollusc likewise fail to stain normally with Janus green B after pretreatment with DFP. The evidence suggests that cholinesterase has a special affinity for Janus green B and that the oriented structures of motor end-plates, referred to as "batonnets" by Couteaux, contain this enzyme.

98. THE EFFECTS OF METHYLENE BLUE PLUS URETHANE (ETHYL CARBAMATE) UPON THE OXYGEN UPTAKE OF EMBRYONIC CELLS. Joseph H. Bodine, State University of Iowa. (Lantern; 15 min.)

Methylene blue added to embryos of the grasshopper, *Melanoplus differentialis*, produces an increase in oxygen uptake. Urethane, within reversible limits, does not inhibit the action of methylene blue. The sequence of additions of methylene blue and urethane, quantitatively affects the oxygen uptake of the embryos. The addition of methylene blue has no effect on the normal respiratory quotient (0.75). Urethane raises the respiratory quotient from 0.75 to 0.94. Urethane and methylene blue mixed and then added to the embryo give respiratory quotients characteristic of methylene blue alone. Possible modes of action of methylene blue and urethane upon the respiratory mechanism are indicated.

99. AN EXPERIMENTAL STUDY OF MITOTIC GELATION. L. V. Heilbrunn, W. L. Wilson and Drusilla Harding, University of Pennsylvania. (Lantern; 15 min.)

The gelation which precedes the appearance of the mitotic spindle in dividing cells is similar to the gelation which occurs in clotted blood. This mitotic gelation can be induced by substances similar to thrombin. Thus when unfertilized sea-urchin eggs are treated with extracts of clotted protoplasm from injured tissues, a high percentage divide. Division is preceded by a pronounced gelation. Also, substances which interfere with the clotting of blood prevent both mitotic gelation and cell division. Dilute solutions of heparin suppress mitotic gelation and mitosis in *Chaetopterus* eggs. A similar effect is produced by the bacterial polysaccharide of Shear. Both ordinary heparin and the bacterial polysaccharide

(which is apparently also a heparin) tend to inhibit the action of blood serum in initiating the division of unfertilized frog eggs. Dicumarol in dilute solutions causes first an enhancement of the mitotic gelation in *Chaetopterus* eggs, but then after several hours the protoplasm becomes extremely fluid. The division of the cells in these solutions is greatly delayed; then typically a single division occurs, with no subsequent divisions. The effect is reversible. Action of vitamin K (2-methyl-1,4-naphthoquinone) is essentially opposite to that of dicumarol.

Apparently all cells contain substances which tend to induce protoplasmic clotting and also substances which tend to prevent such clotting. In the interplay of these substances one can find an interpretation of the factors which induce cells to divide and those which prevent them from dividing. Eventually, knowledge of these factors may make possible a control of abnormal cell division.

100. SOME EFFECTS OF URETHANE (ETHYL CARBAMATE) ON MITOSIS IN THE TROUT BLASTODERM. Helen I. Battle and Margaret A. Laing, University of Western Ontario. (Lantern; 15 min.)

Urethane, a water soluble chemical having some carcinogenic properties, has been shown to inhibit mitoses in the corneal epithelium of the rat, when injected intraperitoneally (Guyer and Claus, 1947). Mitotic stages can readily be differentiated and counted from early cleavage to late blastulation, in the trout egg, which hence serves as a useful experimental medium. Eggs were treated with 0.1 to 1.0% of urethane for periods varying from 6 to 72 hours. With increasing concentrations of urethane, during the early stages, there appears to be an initial decrease in the numbers of interphases and prophases, accompanied by an increasing metaphase count, while the percentages of anaphases and telophases remain fairly constant. As blastulation proceeds a subsequent decrease in all mitotic stages occurs, along with complete inhibition at high concentrations. In 0.1 to 0.75% of urethane where mitoses are not completely arrested, binucleate to multinucleate giant cells as well as multipolar mitotic figures appear in the deeper peripheral regions of the blastoderm.

101. GROWTH FACTOR REQUIREMENTS OF *TETRAHYMENA GELEII* E. A. M. Elliott, Zoology Department, University of Michigan. (Lantern; 10 min.)

This organism can be maintained continuously in a purified medium consisting of 11 amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, and valine), dextrose, sodium acetate, inorganic salts (K_2HPO_4 , $MgSO_4$, $FeCl_3$, $Fe(NH_4)_2(SO_4)_2$, $CaCl_2$, $CuCl_2$, $MnCl_2$, $ZnCl_2$) and several growth factors. Pantothenic acid, nicotinic acid, pyridoxine, and the recently isolated protogen (cf. Stokstad, et al.), are essential for growth; thiamine and choline stimulate growth but are not essential. Cultures appear to be transplantable without folic acid and biotin although as yet this is not certain. Riboflavin is neither essential nor stimulatory. This strain of *Tetrahymena* seems to be able to synthesize both riboflavin and folic acid which is not true of strain W (cf. Kidder and Dewey). Vitamin B_{12} cannot replace any of these growth factors and fails to stimulate when it is added as a supplement to the complete medium.

102. COMPARATIVE AMINO ACID METABOLISM OF *TETRAHYMENA GELEII* E. A. M. Elliott and J. F. Hogg, Departments of Zoology and Biological Chemistry, University of Michigan. (Lantern; 5 min.)

The comparative amino acid metabolism of this organism has been studied by replacing or supplementing several of the 11 growth-essential amino acids (cf. Elliott, 1949) with compounds which are either structurally or metabolically related to the individual amino acids. A growth technique somewhat different from that of others was necessitated by the interesting fact that the organism adapts to growth on simpler media after three or 4 serial transfers in completely synthetic medium. For this reason no more than two growth transfers from a peptone medium have been used. The results differ in several respects from those obtained from similar studies with other organisms, chiefly mammals. For example, phenylalanine is not spared by tyrosine, methionine by cystine, nor histidine by imidazolelactic acid. Growth data on arginine leave open the question of the existence of the urea-ornithine cycle (Krebs and Henseleit). All of the keto analogues tested thus far have replaced the corresponding amino acid to some extent and carnosine has replaced histidine completely. All replacements show a lag phase in growth initiation.

General Morphology, Ecology Evolution and Genetics (Section A)

103. NOTES ON THE RARE ARCHIANNELID GENUS *DINOPHILUS* IN THE UNITED STATES. E. Ruffin Jones, Jr., University of Florida, and Frederick F. Ferguson, University of Washington. (10 min.)

Archiannelids, because of their small size and sporadic occurrence have been little studied in this country. The genus *Dinophilus* is represented by *D. gardineri* which was described from Woods Hole, Mass. and *D. gyrocoliatius*, a European, which has been reported from New Jersey. We have studied two new forms, *D. jagersteni* from Virginia and North Carolina, another Atlantic Coast form and the first in which hermaphroditism is reported; and *D. kincaidi* from Washington, the first form to be reported from the Pacific coast. In addition to a description of the morphology of these forms some discussion of their ecology is given.

104. MOTOR NERVE ENDINGS IN MUSCLE OF THE COCKROACH. Elizabeth A. Weiant, Tufts College. (Introduced by Kenneth D. Roeder.) (Lantern; 15 min.)

Transection of a motor nerve supplying one of the trochantin muscles in the cockroach results in a complete loss of muscle excitability to electrical stimulation about the 5th day after nerve section. Since there are no obvious morphological changes in the muscle at this time, histological studies were made.

Preparations of teased muscle, longitudinal sections, and cross sections were examined. It was found that the majority of the endings were fine threads terminating freely in the muscle without any branch or cone.

In most cases one could predict the point of penetration of a nerve branch into the muscle by a typical indentation which appeared at the muscle surface in a section or two preceding that in which the nerve was actually found penetrating the muscle.

As the nerve branch penetrated the muscle, it divided into a number of fine branches which travelled for some distance across and through the muscle fibers. These branches showed small varicosities and frequently appeared to be double. There were two ways in which these branches terminated. One type went directly through the muscle fibers to innervate on the opposite side of the muscle, while a second type coursed longitudinally between two muscle fibers, to which it gave off a series of terminal branches approximately $40.0\ \mu$ apart. In both types the nerve termination lacked a differential end-plate or brush.

105. RESPIRATORY AREA OF THE TOADFISH. I. E. Gray, Duke University. (Lantern; 15 min.)

The number of gill platelets per gram of body weight or per square centimeter of body surface decreases markedly with increase in size of the fish. The area of gill surface of 53 toadfish averaged 198 sq mm per gram of body weight and 125 sq mm per square centimeter of body surface. The respiratory area of the toadfish is small when compared to that of more active pelagic fishes, such as menhaden and mackerel.

106. THE MORPHOLOGY OF THE LYMPHATICS OF THE RABBIT OVARY, AND THEIR POSSIBLE RELATION TO OVULATION. J. H. Buttr, Jr. and Joseph I. Davies, The Rice Institute. (Introduced by Theo. L. Jahn.) (8 min.)

The number and size of the lymph vessels in the rabbit ovary appear to be disproportionate to the size of the organ. Furthermore, the lymph vessels present an unusual arrangement in that they are directly connected to the blood vessels. Evidence for these connections was obtained from histological studies made on ovaries in which the blood vessels were injected, and also on ovaries which were not injected. In addition, histological studies showed that following copulation the capillaries in the ovary were dilated and engorged with corpuscles indicating a condition of stasis. In some instances the capillary walls were broken down and blood was extravasated into the tissues.

Observations made on living ovaries during the ovulation period showed that the ovaries increased in size, and that towards the end of the period they were considerably swollen, somewhat translucent, and decidedly hyperemic. It was also observed that ovulation could be accelerated 30 or 40 minutes by clamping off all vessels connected to the ovary.

It is suggested that an excessive formation of lymph during the ovulation period and the accumulation of lymph in the ovary during this time, due to partial obstruction of the drainage system, are factors which contribute to the ovulation process.

107. THE AFFINITIES OF THE BRYOZOA. F. H. Pike, Columbia University. (10 min.)

Destruction of the nervous system first formed in embryonic life in Bryozoa, Tunicates and Crinoids, with subsequent imperfect development of somatic musculature, suggests some factor common to all these forms. The clearest indication

of what this factor may be is found in the Crinoid *Antedon*, in which the embryonic nervous system degenerates when the mouth migrates from its initial position in ontogeny. Some close relation between mouth and nervous system in embryonic development seems clear.

Tunicates and Echinoderms are Deuterostomes, but the situation in Bryozoa is uncertain. Origin of Deuterostomes from, or about the stage of, ancestors of Nematomorpha is suggested by conditions in Enteropneusta, in which a possible manner of origin of the vertebrate nervous system seems reasonably clear. Development of the nervous system from the form in which it appears in Plathelminthes through Trematodes and Nemertines to that of Nematomorpha can be traced in living forms. Close nervous connection between dorsal ganglion and anterior region of alimentary tract, (Hubrecht), is present from monogenic Trematodes on. Hubrecht's assumption of homology of dorsal ganglion from Nemertines to vertebrates seems justified.

In view of known facts, it is suggested that Bryozoa separated off from the other line of descent at, or near, the stage of origin of Deuterostomes. No other Deuterostomes are known than those through which ancestry of vertebrates may be traced, provisionally.

108. THE RELATIVE POSITION OF THE CETACEA AMONG THE ORDERS OF MAMMALIA AS INDICATED BY PRECIPITIN TESTS. Alan Boyden and Douglas Gemeroy, Rutgers University. (Lantern; 15 min.)

The systematic position of the Cetacea among the orders of Mammalia is uncertain. In order to obtain further data bearing upon this problem we have compared the sera of representatives of the Cetacea with those of 15 other orders of mammals. Sixty-five precipitin antisera, representing 14 orders have been tested with the sera of all other existing orders of mammals except the Dermoptera and Lagomorpha. The interordinal reactions as determined by the quantitative photoelectric technique using the Libby photoreflexometer were generally weak, averaging about 2%. But the tests between Artiodactyla and Cetacea were distinctly higher averaging about 11%. These tests indicate a greater similarity between the proteins of the sera of representative whales and even-toed ungulates than between either of these orders and any other orders tested. The tests revealed no significant effect of the removal of lipid materials from the antigens upon the relationship values obtained. Antibovine sera containing high proportions of antialbumins reacted more strongly with whale sera than did those antibovine sera containing low proportions of antialbumins. These results indicate that the albumins of these mammalian sera are more similar than their globulins. Thus a new source of variability in the reactions of antisera to native sera has been disclosed and attention must hereafter be given to the relative proportions of antialbumins and antiglobulins in the antisera used. The evidences warrant the conclusion that the Cetacea have a closer systematic relationship to the Artiodactyla than to any of the other mammalian orders tested.

109. STUDIES IN THE PHYSIOLOGY OF COMMENSALISM. I. THE POLYNOID GENUS *ARCTONOE*. Demorest Davenport, University of California, Santa Barbara College. (15 min.)

Members of the genus *Arctonoe* are commensal in the ambulacral grooves of certain starfish, on the surface of holothurians and in the mantle cavity of certain gastropod mollusks. The worms exhibit a marked species specificity.

Apparatus was constructed which made it possible to give the commensals a choice between sea-water from an aquarium containing their host and sea-water alone or sea-water containing non-host. Statistically significant samples showed that the worms chose the host path in the great majority of tests. There is evidence that whatever the diffusible substance may be that the worms sense from their hosts, it is very specific. Commensal *Arctonoe* from the star *Evasterias troschelii* and from the Holothurian *Stichopus californicus* both respond positively to their own hosts but not to each other's.

110. DIFFERENCES IN TOLERATION OF DRYING BETWEEN SPECIES OF TERMITES (*RETICULITERMES*). Margaret J. Strickland, The University of Chicago. (Introduced by Harold E. Finley.) (5 min.)

Three species of the genus *Reticulitermes* of the Chicago area were compared in their resistance to drying. This resistance was measured in average survival time of groups of 50 termites exposed to a flow of air dried to 0-2% relative humidity, at 34°C.

Environmental factors were found to affect survival time to a marked extent, and since the three species inhabited different areas, with correspondingly varied environmental conditions, a controlled pre-experimental environment had to be utilized before comparing average survival time of one species with that of another. The search for an optimal pre-experimental environment led to an investigation of some of the factors involved in resistance to drying. Abrasions of the body by sand particles, observable cuticular differences (as between imagoes and the other castes), and size (surface-volume ratios) were found to be negligible in affecting survival time. Optimal pre-experimental conditions were obtained by keeping the termites in finger bowls containing a mixture of agar and sawdust in the incubator at 34°C. It is probable that variation in the amount of fluid in the gut is the factor responsible for the variation in resistance between different colonies of the same species, and different samples from the same colony.

Of the three species tested *R. tibialis* showed the greatest resistance, *R. flavipes* was intermediate, and *R. arenincola* showed the least resistance to drying. There was a correlation between resistance to drying and ecological distribution for two of the species.

111. A CASE OF CYTOPLASMIC TRANSMISSION IN *DAPHNIA PULEX* DEGEER. A. S. Bradshaw, Cornell University. (Introduced by D. C. Chandler.) (10 min.)

Starvation was employed in investigating the possibility of the cytoplasmic transmission from parent to offspring of the effects of an unfavorable environment. Offspring from starved mothers and offspring (controls) from well fed mothers

of the same genetic line were compared under starvation conditions (simultaneous, highly dilute cultures). The average length of life and the average number of young produced by the offspring from starved mothers were significantly greater than that shown by offspring (controls) from well fed mothers.

112. IMPROVED AXENIC CULTIVATION OF RHABDITIS BRIGGSÆ DOUGHERTY AND NIGON, 1949 (NEMATODA). Ellsworth C. Dougherty,¹ University of California (Berkeley) and California Institute of Technology, James C. Raphael, Jr., and Carlota H. Alton, University of California (Berkeley). (Lantern; 7 min.)

It has recently been shown (Dougherty, E. C., *Science*, in press) that *R. briggsæ* (which is genetically more favorable than *R. pellio*) can be grown indefinitely on sterile pieces of chick embryo, although maturation is less rapid than in the presence of suitable bacteria. Subsequent work has revealed that a medium made up with most of the ingredients used for *Tetrahymena* by Kidder and Dewey (*Arch. Biochem.* 20: 433-43, 1949—for amino acids, salts, and tween; *J. Nutrition* 37: 521-9, 1949—for other ingredients) and at the same levels as therein (with the following more or less arbitrary exceptions—in γ /ml: Ca pantothenate, nicotinamide, and riboflavin, 1.0 [*vs.* 0.1]; pteroylglutamic acid, 0.1 [*vs.* 0.01]; biotin [methyl ester], 0.05 [*vs.* 0.0005]; protogen, 0.5 [*vs.* 0.375]; no guanylic or adenylic acid [*vs.* 30 and 20 resp.]; thymine, 50 [*vs.* none]) would support growth superior to that with chick embryo alone when Kidder-Dewey ingredients were autoclaved at double strength in aqueous solution and then diluted to standard strength with chick embryo juice prepared by sterile pressing of approximately 12-day chick embryos from which eyes had been removed. Growth was about as rapid and reproduction as abundant as in the presence of bacteria. Nothing as yet can be said about absolute requirements for the defined ingredients used, but this medium does provide an adequate basis for studies on the isolation of the heat-labile factor(s) in chick embryo juice.

¹ Senior Research Fellow of the American Cancer Society as recommended by the Committee on Growth of the National Research Council, 1949-52.

113. ATTEMPTS AT HYBRIDIZING TWO CLOSELY RELATED HERMAPHRODITIC SPECIES OF THE NEMATODE GENUS RHABDITIS. Victor Nigon, Faculté des Sciences P.C.B. (Paris); and Ellsworth C. Dougherty,¹ University of California (Berkeley) and California Institute of Technology. (Lantern; 8 min.)

Attempts were made to hybridize the two species, *Rhabditis elegans* Maupas, 1900, and *R. briggsæ* Dougherty and Nigon, 1949 (*J. Parasitol.*, vol. 35, pt. 2) by reciprocal matings of males and hermaphrodites of the two species. (For general methodology of work with free-living nematodes, see: Nigon, V., *Ann. Sci. Nat.*, vol. 11, pp. 1-132, 1949). These attempts uniformly failed as judged by: (a) failure of an increase in the *andric index* of the progeny of the hermaphrodites so mated; (b) failure of transmissal of a recessive mutant gene present in *R. briggsæ* from the males to succeeding generations as judged by failure of its phenotypic manifestation in the F₂ (where it would be expected to appear if hybridization had succeeded); and (c) failure to obtain intermediate

morphological types. All evidence points to the morphological and functional separation of the two species. Nevertheless, *R. elegans* and *R. briggsae* are morphologically very similar, with similar chromosome patterns ($2n = 11$ or 12), behavior patterns, and reactions to elevated temperatures ($23-25^{\circ}\text{C}.$), *i.e.*, induced meiotic anomalies. The genus *Rhabditis* as now constituted contains over 100 described species. Some of these are very likely identical, but the study reported here suggests that there may be pairs, or even large groups of closely related species, at the present time regarded as single species. Much careful comparative work seems indicated, making use of cultures raised under standardized conditions.

¹ While in France: Fellow of the John Simon Guggenheim Memorial Foundation, 1947-49, and U. S. National Cancer Institute Postdoctorate Fellow, 1947-48. At present: Senior Research Fellow of the American Cancer Society as recommended by the Committee on Growth of the National Research Council, 1949-52.

114. INTRA-UTERINE DEATH TIME IN SEMI-STERILE MICE. Eileen M. Otis, University of Rochester. (Introduced by Donald R. Charles.) (Lantern; 15 min.)

Semi-sterile mouse pedigrees descended from males exposed to chronic x-radiation have been studied to determine intra-uterine death time of inviable zygotes. Inviability is presumably due to unbalanced chromosomes. Six pedigrees show developmental failure before the 12th day of pregnancy.

Comparison of mouse and human developmental stages has been made using the literature on human development and timed, serially-sectioned mouse embryos (7-17 days of pregnancy). A curve has been constructed by plotting the time of appearance and differentiation of individual organs or tissues in mouse embryos against the time of corresponding structures in human embryos. From this curve, it is judged that the time span of intra-uterine failure (7-12 days of mouse pregnancy) in the present sample of semi-sterile pedigrees is probably comparable to the second to 6th week of human pregnancy.

General Demonstrations (Section B)

115. X-RAY INDUCED GREYING IN THE MOUSE WITH REFERENCE TO MELANOBLASTS. Herman B. Chase and Virginia W. Smith, Brown University.

After exposure of colored mice to x-radiation, a certain percentage of follicles produce white hairs in all subsequent hair generations. The percentage of follicles which respond in this manner depends on the radiation dose and dose-rate, stage of hair growth at the time of treatment and the size of follicle (hair type). Mosaic hairs induced by x-radiation are relatively infrequent in the mouse. In later hair generations these follicles produce either mosaic or white hairs with a progressive and irreversible tendency toward white.

Histological methods, especially phase contrast microscopy on frozen sections, reveal certain pertinent details of pigmentation. At 3 and 4-day post-plucking hair growth stages, the melanoblasts which are to supply that hair generation contain fine melanin granules and possess extensive dendritic processes. By 6

days the "inoculation" of the matrix cells by these processes begins. At the 7- to 8-day stage the skin is macroscopically darkened. In newborn mice, the more advanced follicles are already equal to the 4-day plucked stage and younger invaginating follicles clearly show melanoblasts migrating down the external sheath from the epidermis. It is probable that only 4 to 8 melanoblasts supply each follicle per generation. Mosaic hairs have only one, occasionally two, melanogenic melanoblasts. Only matrix cells in contact with melanin-bearing dendritic processes become pigmented. Apparently, some genetic variations in color are imposed by matrix cells on the granules received. The melanoblast "reservoir" for each follicle is not dermal but is associated with certain regions of the permanent external sheath.

116. HAIR GROWTH AND PIGMENTATION IN RELATION TO BIOTIN DEFICIENCY. Harold Rauch, Brown University. (Introduced by Elizabeth B. Chase.)

C57 Black mice (16-20 days) are weaned to a diet resulting in biotin deficiency. One month later, early symptoms are evident (loss of hair and hair color). Plucking during early deficiency is followed by new hair growth, lightly pigmented. Even in advanced deficiency, normal waves of growth can be observed, but completed hairs are not maintained. Administration of a single dose of biotin (2 gamma) has no effect on waves of growth, which proceed at normal rates, but has a striking effect on color. Within 24 hours, there is darkening of skin in the wave only, due to the appearance of dark pigment in these active follicles. The first hairs to emerge from this dark region are light at tips and dark at bases, followed by hairs further along the wave which are entirely black and still further by hairs dark only at the tips. Finally only light hairs occur. This single dose of biotin is not sufficient to cause retention of hairs after completion of growth.

These observations suggest that biotin is necessary neither for initiation of hair growth nor for hair formation, but is necessary for hair retention and normal club formation. Further, biotin is needed for a final step in the pigmentation process. A single small dose allows normal pigmentation to be maintained for about 10 days. Portions of the shaft already developed remain light, whereas those developing during this 10 day period become dark. Later portions are again light.

117. DEHYDROGENASE ACTIVITY OF NORMAL AND NEOPLASTIC TISSUE AS INDICATED BY THE REDUCTION OF THE TRIPHENYL TETRAZOLIUM CHLORIDE. Maurice M. Black, M. D., New York Medical College. (Introduced by Secretary.)

Tissue slices from a variety of human organs and tissues were incubated in a solution of 2, 3, 5 triphenyl tetrazolium chloride and the relative amounts and localization of the red formazan were noted. With this technique it was found that:

(1) Triphenyl tetrazolium chloride is actively reduced by most epithelial elements of most tissues but to a much lesser degree by normal lymphoid or fibrous tissue or by inflammatory tissue.

(2) Malignant tumors arising from epithelial structures tend to retain the high dehydrogenase activity of the parent tissue.

(3) The high degree of contrast between the colored malignancy and the unstained stroma or lymph node provides an excellent topographic demonstration of tumor spread.

The ability of various tissue components of different organs to reduce triphenyl tetrazolium chloride will be demonstrated.

Representative examples of normal and neoplastic human tissue will be shown.

118. STRUCTURAL MAKEUP OF TERMINAL VASCULAR BED. Benjamin W. Zweifach, Department of Medicine, Cornell University Medical College.

The regulation of the flow of blood through the terminal blood vessels is in large part dependent upon the peculiarities of the structural makeup of the capillary bed. In tissues such as the mesentery, skin and skeletal muscle of the rat the peripheral blood vessels beyond the arterioles are not haphazardly distributed but consist of groups of functional units, each built around a centrally placed muscular channel from which capillary side branches are given off. The arrangement of the vessels is such that in the normal resting state the peripheral circulation is restricted to preferential capillary channels, the metarterioles. These vessels, which continuously convey an active flow of blood, are distinguished by occasional thin branched, poorly contractile muscle elements in their walls. The remainder of the capillary vessels are interposed as side branches which are given off from the proximal portion of the metarterioles and then drain into the distal or venous segment of the same vessel. They are denuded endothelial tubes which possess no contractile pericapillary elements and are termed "non-muscular" or "true capillaries." A significant feature of the capillary offshoots is the presence of one or two muscle cells just as the branch leaves the parent vessel. The term "precapillary sphincter" has been applied to this strategically placed junctional muscular portion of the capillary, the state of contraction of which directly controls the blood flow into the capillary network.

119. THE EFFECT OF INJECTION DOSAGE LEVEL OF LYOPHILIZED MOUSE TISSUE ON THE SUBSEQUENT GROWTH OF A TUMOR HOMOIOTRANSPLANT.¹ Nathan Kaliss² and Olive Newton, Roscoe B. Jackson Memorial Laboratory.

Prior injections of lyophilized tumor tissue may produce either inhibition or enhancement of growth of tumor homoiotransplants in mice (Snell et al, 1946, 1948). In an attempt to elucidate the mechanism, or mechanisms, whereby these results are produced, we studied the effects of different dosage levels of lyophilized mammary adenocarcinoma Eo 771 on the subsequent growth of this tumor in strain C₃H Jax mice. Tumor Eo 771 is indigenous to strain C57 black, but will give positive takes in the unrelated C₃H Jax mice, killing about 50% of the recipients. Dosages varied through a 10-fold serial dilution from a total of 50 mg down to 0.05 mg per mouse of dry weight of lyophilized tumor. The following results were obtained, with the various total dosages per mouse as indicated: 0.05 mg,

15 out of 19 mice (79%) negative; 0.5 mg, 6/19 (32%) negative; 5.0 mg, 5/19 (26%) negative; 50.0 mg, 1/17 (6%) negative; control (no prior injections), 9/17 (59%) negative. Thus, very small doses of lyophilized tumor apparently have an inhibitory effect, while larger doses have a growth enhancing effect on tumor homoio-transplants.

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²Senior Fellow in Cancer Research of the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

120. A STUDY OF ANTIBODY PRODUCTION IN INBRED STRAINS OF MICE IN CONNECTION WITH THE GROWTH OF TRANSPLANTED TUMORS.¹ George E. Jay, Jr. and Nathan Kaliss,² Roscoe B. Jackson Memorial Laboratory

An attempt has been made to correlate the development of an agglutinin titer during the course of injections of lyophilized tissue (normal and tumorous), with subsequent growth of a homoio-transplant. Experiments using lyophilized A strain normal (liver, kidney, red blood cells, spleen) and tumorous (spindle cell carcinoma 15091a) tissue inoculated into C57 black subline 6 animals have given evidence that if a correlation exists, it is not clear-cut.

Agglutinin titers were followed using (1) pooled serum from approximately 20 animals in each group, and (2) serum from individual animals.

Lyophilized A strain liver, kidney, and red blood cells failed to produce measurable agglutinin titers, and subsequent growth of homoio-transplants was not consistent. In all groups, some animals showed progressive growth while others showed no growth at all. Lyophilized A strain spleen and tumor 15091a produced measurable titers, but again some animals showed progressive growth of homoio-transplants while others did not.

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²Senior Fellow in Cancer Research of the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

121. STAINING OF NUCLEIC ACIDS BY AZURE A. Martin Flax and Arthur W. Pollister, Columbia University.

Experiments indicate that the basic dye Azure A. (Nat. Anil., Cert. No. NAz 14), when used in 0.1% aqueous solution and differentiated overnight in absolute ethyl alcohol, is a specific stain for nucleic acids. In nerve cells, several epithelial tissues, and various embryonic structures the stain is localized in chromatin, in nucleoli, and in cytoplasmic regions, which ultraviolet absorption and protein tests show to be sites of high nucleoprotein concentration. This

stainability with Azure A is entirely destroyed if the nucleic acids are removed by nuclease digestion or extraction with hot trichloroacetic acid. The method fails to stain regions low in nucleic acid, such as thyroid colloid, muscle cytoplasm and the cytoplasm of erythrocytes. An exception to this general specificity for nucleic acid is that Azure A stains granules of mast cells, mucus, and cartilage matrix, but this is readily distinguishable from the nucleic acid staining since it is not destroyed by ribonuclease and the color tends to appear metachromatic. Absorption measurements show that under identical conditions of fixation and staining the variations in intensity on different slides of one tissue fall within the range of error of the photometric technique. The stain intensity can be varied considerably by changing the fixation, pH of the stain, or nature of the decolorizing alcohol. A preliminary quantitative comparison of ultraviolet absorption and staining with Azure A suggests that if the staining process is chemical, the dye-nucleate compound represents only a fraction of the total pentose nucleic acid of the cell.

122. PHOTOMETRIC ANALYSIS OF CELLS. Arthur W. Pollister, Montrose J. Moses and Cecile Leuchtenberger, Columbia University.

The construction and operation of devices for making transmission measurements of small areas of cells will be demonstrated. Specific methods for determining relative amounts of proteins and nucleic acids in cells, by measuring either the natural absorption of ultraviolet light or the absorption by various color reactions of these substances, will be described by charts.

123. A HISTO-PHOTOELECTRIC METHOD OF COLLAGEN DETERMINATION IN MUSCLES.

Hsi Wang, American Meat Institute Foundation, The University of Chicago.

Collagen is determined conventionally by chemical analysis.¹ This method requires fairly large samples, and does not indicate the distributional pattern of the collagenous fibers in meat. The present technique was devised to estimate the collagen content in histological sections. Ten-micron sections of beef are stained with Van Giessen's mixture of picric acid and acid fuchsin long enough to render muscle fibers yellow and collagenous fibers red over a yellow base. No nuclear stain is used. A photoelectric densitometer² is used to measure the optical density of these stained sections. It employs a photoelectric cell (probe) which is highly sensitive to the green absorbed by the stained collagenous fibers, and the rays so transmitted by the probe are greatly amplified before reaching a built-in galvanometer calibrated on a scale from zero to 200. Readings are taken under the high power of a binocular compound microscope with the probe placed over one of its eyepieces (the other for observation during measurement), and spaced by mechanical stage at one reading per square millimeter. These are entered under three main categories according to the structure in the field of measurement: (1) practically all muscle fibers, (2) muscle and collagenous fibers, and (3) collagen and fat, or collagen, muscle fibers and fat. The per cent collagen by volume present in the measured section is computed by this formula:

$$\frac{\left(\frac{\sum n_2}{n_2} - \frac{\sum n_1}{n_1} \right) \times (n_2 + n_3)}{NK}$$

Where, $N = n_1 + n_2 + n_3$; K = density of pure collagen per reading; n_1 = No. type (1) readings with their sum density, Σn_1 ; n_2 = No. type (2) readings with their sum density, Σn_2 ; n_3 = No. type (3) readings with their sum density, Σn_3 . One example which gave the following data: $n_1 = 58$; $\Sigma n_1 = 859$; $n_2 = 72$; $\Sigma n_2 = 1268$; $n_3 = 17$; $\Sigma n_3 = 291$; $K = 80$, is found to contain 1.8% of collagen.

¹ Lois N. Hartley and J. L. Hall, 1949. Food Research, 14: 195-202.

² Densichron, made by Welch Scientific Co., Chicago.

124. DEMONSTRATION OF TISSUE SECTIONS PREPARED BY RAPID FREEZING-SECTIONING TECHNIQUE. A. B. Taylor and F. B. Adamstone, University of Illinois.

Histological and cytological preparations of rat tissues made from fresh tissues by the rapid freezing-sectioning technique developed by the writers ('48) will be on demonstration, including:

Fat distribution in liver.

Mitochondria in liver cells (Altmann preparation).

Feulgen preparation.

Gomori preparation for alkaline phosphatase.

125. THE EFFECT OF ADDED DIETARY TRYPTOPHANE ON THE OCCURRENCE OF DIETHYLSTILBESTROL INDUCED MAMMARY CANCER IN THE RAT. W. F. Dunning, M. R. Curtis, and M. E. Maun, Detroit Institute of Cancer Research, Department of Pathology, Wayne University College of Medicine, and St. Mary's Hospital, Detroit.

Three groups of A × C line 9935 female rats with diethylstilbestrol pellets implanted in their scapular region, were placed on iso-calorie synthetic diets containing 26% protein. The first group of 12 received the diet with 26% casein, the second group of 12 received the same diet, substituting 24.6% tryptophane-free casein and 1.4% d-l tryptophane for the intact casein and the third group of 15 received in place of the intact casein, 21.7% tryptophane-free casein plus 4.3% d-l tryptophane.

All 39 rats lived more than 160 days, the minimum period before the occurrence of an induced mammary cancer, and 32 or 82% developed multiple mammary cancers. Of the control group, 9 or 75% developed 51 gross cancers in an average of 363 ± 8.6 days, while 100% of those which received 1.4% d-l tryptophane developed 79 gross mammary cancers in an average of 316 ± 5.7 days and 9 or 60% of the rats that received the high-tryptophane diet developed only 31 gross mammary cancers in an average of 450 ± 14.5 days.

The addition of 1% d-l tryptophane increased the number and percentage of induced cancers and significantly reduced the average latent period below that observed for the control group. The greater increase in dietary tryptophane produced the reverse effect of a decreased number and percentage of induced cancers and a significantly prolonged latent period.

126. THE IMMEDIATE SOURCE OF THE PRIMARY INTERSTITIAL TISSUE OF THE OVARY OF THE INFANTILE RAT. Alden B. Dawson, Harvard University.

Previously it was reported (Dawson and McCabe, 1948) that primary interstitial tissue which becomes obvious at 8 days after birth is locally cholesterol-positive at 10 to 12 days. It appears as irregular areas in the interfollicular stroma, not directly related to the primitive thecae which at this stage show no differentiation into externa and interna. Definitive thecae appear at 18 days or later, the interna becoming histochemically-positive and giving rise to a secondary interstitial tissue during ensuing atresia. Many of the cord-like masses of primary interstitial tissue are continuous with granulosa cells of the follicles. As many as three follicles may be united in series by such cords but more frequently these extrafollicular extensions end blindly. Only the extrafollicular tissue becomes histochemically-positive. These patterns may result directly from a persistence of the original ingrowing cords from the germinal epithelium but the evidence is not conclusive. Extrafollicular cords become most apparent during first antrum formation when the follicles are increasing rapidly in volume. It is suggested that these extrafollicular extensions are produced, in part at least, by intrafollicular pressure, resulting in local rupture of the follicular capsule and permitting the escape of granulosa cells into the ovarian stroma. Later due to structural rearrangements occasioned by continued follicular growth the masses of primary interstitial tissue become isolated, flattened, and secondarily, closely applied to the follicular surface, thus simulating a histochemically-positive, discontinuous theca which is separated from the follicular capsule by the non-reactive primitive theca.

127. DEMONSTRATING AMPHIBIAN CLEAVAGES WITH DRY MOUNTS. Mychyle W. Johnson, Duke University.

Some of the difficulties encountered in use of fluid-preserved material for class demonstration are eliminated by employing dried specimens mounted on plastic strips. These are permanent and easily oriented in any position. Procedure: wash in water; remove membranes; bleach; repeat washing; dehydrate and place in xylene; air dry; fasten to plastic strips with adhesive.

This method is especially useful for early cleavage but can also be applied to older stages.

128. PATTERN FORMATION IN A BERMUDA CORAL REEF FISH. H. B. Goodrich, Ruth L. Hine and Jack Reynolds, Wesleyan University.

Experiments were made on *Halichoeres bivittatus* (Bloch), a Bermuda coral reef fish. It is marked by longitudinal dark and light stripes. Autoplastic tissue exchanges by means of scale transplantation were made between the two stripes. Within a few weeks melanophores invaded white scales placed in the dark stripe and degenerated on dark scales placed in the white stripe. Similar transplants were made on fish subsequently kept in the dark, on a dark background and on a light background in sunlight, and on blinded fish kept in the dark and in sunlight. The results indicate that under all these conditions melanophores

degenerate in transplants from the dark to the light stripe. In transplants from the light to the dark stripe the melanophores increase most rapidly in bright sunlight, regardless of background, and less rapidly in diffuse light and in the dark and on blinded fish in sunlight but not at all on blinded fish in darkness. It appears that conditions in the light stripe are unfavorable for melanophores and there is no source of replacement. In the dark stripe there is a source of replacement. The condition of bright sunlight which characterizes the normal environment of the fish appears to be that most favorable for the maximum development of the color pattern. It, however, is not clear that this is mediated by visual stimuli.

129. NEW MUTATIONS AND LINKAGE STUDIES IN THE HOUSE MOUSE (*MUS MUSCULUS*).¹ M. M. Dickie, D. E. Kelton, J. H. Fielder, A. M. Ingalls and G. D. Snell, Roscoe B. Jackson Memorial Laboratory.

A dominant mutation, alopecia (*Al*), found in the fuzzy stock, acts similarly to naked; however, homozygous alopecias regain all of their hair for brief periods. During molt, heterozygotes lose ventral surface hair only while homozygotes lose all except guard hairs.

Wabblers-lethal (*wl*), a recessive mutation, occurred in the pirouetter stock. Males die about three weeks, females about 6 weeks of age. Wabblers animals seem to have a partial paralysis of their hind quarters that makes them quiver and stumble as they move. They are not deaf. Heterozygous wabblers-lethal animals can be classified by a slight waddle in their walk.

A recessive coat color mutation, resembling ruby, has been noted in strain C57 black line 10. The phenotype differs from ruby in that the belly is lighter, with a distinct yellow at the margins, and in that the eye color is not affected.

The recessive mutation ducky (*du*) affects the hind legs causing them to spread at a greater angle from the body. Balance of older animals is upset; in walking they tend to waddle and to fall on their side. Fertility is reduced, particularly in females, which seldom breed. The F_2 ratio is 130 *Du* : 32 *du*.

Linkage between splotch (*Sp*) and short Danforth (*Sd*) has been found. Crossover percent for males is 25.53 ± 7.3 , for females 38.78 ± 7.1 .

¹This work has been supported by grants to the Roscoe B. Jackson Memorial Laboratory from the Commonwealth Fund and the National Cancer Institute.

130. CYCLIC CHANGES IN THE EPITHELIUM OF THE VAGINA, CERVIX, AND UTERINE BODY OF THE GOLDEN HAMSTER CORRELATED WITH VAGINAL SMEARS AND THE EFFECTS THEREON OF CASTRATION AND ESTRONE ADMINISTRATION. George C. Kent, Jr. and J. Harvey Roberts, Louisiana State University.

The epithelia of the upper vagina, uniluminate and biluminate portions of the cervix, and the paired canals of the body of the uterus undergo cyclic changes. A foliaceous epithelium is exhibited at these levels at the time the vaginal smear exhibits large, scale-like squamous cells alone. The latter arise by non-cyclic sloughing of the epithelium of the lower vagina and vaginal pouches, which regions remain constantly cornified. Metestrus is characterized by extensive

exfoliation of the foliaceous epithelium, a condition reflected in the vaginal smear by the appearance of large numbers of elongated epithelial elements accompanied shortly by other epithelial cells including columnar, cuboidal, and oval types. During diestrus a stratified squamous epithelium predominates in the cyclic regions, no extensive foliaceous elements being present. Numerous leucocytes are found internal to, transmigrating through, or near the surface of the epithelia, and in the lumina of the tracts. During diestrus leucocytes occur in vaginal smears along with epithelial elements, although elongated epithelial cells (mostly remnants of earlier exfoliation) have been largely eliminated from the system. In late diestrus and proestrus the epithelium becomes increasingly foliaceous. The concomitant proliferation causes epithelial elements gradually to disappear from smears with the exception of squamous elements from the lower vagina. The castrate condition is reminiscent of, but not identical with, that in diestrus. Prolonged administration to castrates of estrone causes cornification of superior levels not cornified during normal cycles.

131. MATURITY INDICATORS IN THE KNEE OF THE RABBIT. Dorcas D. Crary, Roscoe B. Jackson Memorial Laboratory. (Introduced by P. B. Sawin.)

Comparison of development of the limb bones of humans and inbred races of rabbits shows a high degree of similarity. Although order of appearance of the epiphyses is not the same, progress of development, once ossification has begun, is similar. The animals used were Sawin's Race X, a subline of Castle's small A race, and maintained in the heterozygous condition for the dwarf gene (*dw*). The normal animals weigh approximately 2290 gm average adult body weight and the heterozygotes approximately 1630 gm. No significant difference was noted in the time at which the various indicators appeared. The *dw* gene, therefore, affects rate of growth rather than total time to maturity.

The charts consist of a series of prints from the X-rays of the distal femur, proximal tibia, and proximal fibula with descriptions of each stage from onset of ossification to maturity; age at which they occur; and diagrams indicating the outstanding changes. The value of the rabbit material lies in the high genetic uniformity and the opportunity which it affords for getting at basic genetic factors which can influence humans as well as rabbits.

132. A STUDY OF THE CENTRAL NERVOUS SYSTEM OF FLIGHTLESS DROSOPHILA MELANOGASTER. Maxwell E. Power, Kenyon College, Gambier, Ohio.

In order to determine the relation between aberrant behavior patterns and the central nervous system 9 mutant strains of *Drosophila melanogaster* which have wings but do not fly were chosen for neurological study. These mutants were: cut, curved, Curly, Lyra, curled, aristapedia, bithorax, taxi, and Stubble; and they were studied in silver and gold impregnated sections. With the methods used the investigation has failed to reveal any detectable changes in the morphology of the central nervous system which can be correlated with the absence of the flight reflex. The known cortical neurons are present, the fiber pathways and glomeruli appear normally developed in the neuropile, and the pair of giant

fibers seem not to be modified in any of the mutant lines. Because flight is a thoracic activity volumetric measurements were made on the thoracic-abdominal nervous system but these quantitative studies likewise failed to demonstrate significant differences between the controls and the experimentals in respect to the volume of the entire thoracico-abdominal nerve mass, that of the cortex, or that of the neuropile. Differing from alterations in peripheral sensory fields, it would appear thus that these genetic deviations in motor behavioral expression are not associated with identifiable aberrations in the central nervous system. No attention has been given to physiology, musculature, skeleton, or peripheral nerves.

133. METHODS OF CULTURING CARNIVOROUS PROTOZOA UNDER BACTERIA-FREE CONDITIONS. Daniel M. Lilly, St. John's University.
(See abstract of paper no. 69 to be read.)

134. ON BINARY FISSION IN SPIROSTOMUM AMBIGUUM. Harold E. Finley and Avery W. Beverley, Howard University.

Normal binary fission was studied with the aid of the Feulgen reaction and hematoxylin stains. Then attention was given to macro- and micronuclei in specimens exposed to the fumes of allyl isothiocyanate.

During normal fission the macronucleus undergoes a decided reorganization which involves (1) the loss of its lobed identity; (2) migration anteriorly; (3) contraction into a rounded mass; (4) migration toward the center of the organism; (5) elongation prior to separation of the daughter organisms and a slow resumption of its lobed identity after the separation. The micronuclei multiply as a result of mitosis; as a rule, they remain in the area adjacent to the macronucleus, being moved anteriorly and posteriorly as the fission process evolves and in harmony with the movements of the macronucleus. Micronuclear reorganization was followed in whole mounts and in sectioned material. Polar cones and spindles were observed but chromosomes were too small to be counted accurately. Approximately equal numbers of micronuclei seemed to be distributed to daughter cells.

The striking morphological effects of allyl isothiocyanate upon the macronucleus, observed by Finley and Wanza (1948), were confirmed. Significant effects of a.i., upon micronuclei were not detected.

It appears that the micronucleus is morphologically less responsive to a.i., than the macronucleus.

135. AN EXTRA POSTZYGOTIC NUCLEAR DIVISION IN PARAMECIUM CAUDATUM. William F. Diller, Department of Zoology, University of Pennsylvania.

In a number of races of *Paramecium caudatum* evidence has been found of an extra postzygotic nuclear division involved in the formation of the definitive nuclei. The fertilization nucleus divides initially to produce a viable nucleus and an inviable sister which quickly degenerates. By means of three subsequent divisions the final 8 nuclei, from which the reconstituted complex is differentiated,

arise from the viable nucleus. Thus, 4 postzygotic divisions occur. The two members of a pair usually separate while the synkaryon is in a late mitotic stage of the first division, but separation may be precocious or it may be delayed until after the time when one of the first postzygotic division products has begun to degenerate.

136. THE EFFECT OF ULTRAVIOLET LIGHT UPON THE STRUCTURE OF THE MACRONUCLEUS OF *PARAMECIUM AURELIA*. R. F. Kimball, Biology Division, Oak Ridge National Laboratory.

The macronucleus of *Paramecium aurelia* can be seen to contain numerous, more or less spherical, dark bodies when examined with the Zeiss phase contrast microscope. In addition, numerous very small, possibly rod-shaped bodies can be seen when the macronucleus is broken by compression. The larger, spherical bodies undergo a series of changes when the animals are exposed to a dose of ultraviolet sufficient to cause death within two days. The changes have been obtained with monochromatic ultraviolet of both wave lengths 2804 Å and 2650 Å, the former being somewhat more effective.

Within an hour after irradiation, the bodies start to clump and become more distinct with phase contrast. They fuse together and vacuoles appear in the fused masses. Finally one or a very few large vacuolated masses are present. If the macronucleus is broken by compression at this stage, the very small bodies can be seen to be distributed uniformly over the whole macronucleus. No clear changes in micronuclear structure have been seen. It seems possible to interpret the larger bodies as nucleolar material and the smaller bodies as chromosomes though this cannot be fully demonstrated as yet. It is of interest to note that animals which have reached the stage in which there is a single large body in the macronucleus can survive in this condition for at least a day. Even such a gross disturbance of nuclear structure is not immediately lethal.

137. EXTRUSION OF NUCLEAR MATERIAL IN IRRADIATED AMPHIBIAN OVOCYTES. William R. Duryee, National Cancer Institute.

Microscopic and photomicrographic illustrations of nuclear extrusions in eggs of *Triturus iridescens*. Also photomicrographs of cytoplasmic injection effects on the nuclear colloids.

138. TRANSFER OF I^{131} , TO THE NURSING MOUSE. Roberts Rugh, Columbia University.

Iodine is very rapidly absorbed by the living organism, whether it is painted on the skin or ingested. I^{131} , when injected subcutaneously (dorsally) into the adult mouse is quite rapidly excreted but when the mouse is nursing the I^{131} passes, instead of through the kidneys, through the mammary glands within 8-10 minutes into the nursing young. This can be demonstrated qualitatively with the Geiger-Muller counter. Changes in the histology of the thyroids of the nursing young will be demonstrated.

139. LIVING CIRCULATION IN THE MESENTERY OF A RAT. B. W. Zweifach, The New York Hospital.

A demonstration under the microscope of the living circulation in the mesentery of the rat, accompanied by several explanatory photographs and charts.

140. VITAL STAINING. Leonor Michaelis (deceased), Member Emeritus Rockefeller Institute.

In memoriam demonstration of Janus Green as a cytological stain of which Dr. Michaelis was the originator.

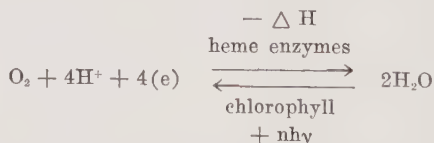
141. THE MORPHOLOGICAL BASIS FOR THE DIAGNOSIS OF CANCER BY EXFOLIATIVE CYTOLOGY. George N. Papanicolaou, Cornell University Medical College. (Introduced by Secretary.)

A demonstration showing, in a series of colored transparencies, the characteristics by which normal and cancer cells can be recognized in smears prepared from various body fluids. Typical smears from various cases will be available for microscopic examination.

142. THE STRUCTURAL AND FUNCTIONAL RELATIONSHIPS BETWEEN THE RED AND GREEN PIGMENTS OF PROTOPLASM. S. Granick, The Rockefeller Institute for Medical Research. (Introduced by Secretary.)

Using chlorella mutants, certain key steps in the biosynthesis of chlorophyll have been discovered. In the scheme proposed for biosynthesis, protoporphyrin isomer no. 9 is the compound from which two branches arise. One branch arises by insertion of Mg into the porphyrin and from this chlorophyll is produced eventually. The other branch arises by insertion of Fe into the porphyrin and the resulting iron porphyrin (or heme) serves as the prosthetic group of heme enzymes and of hemoglobin.

The functional relation between the red and green pigments is made apparent if we write the general energy equation of protoplasm in the following way:



The forward reaction represents the process of oxidation in which O_2 , protons (H^+), and electrons (e) of high potential energy and representing the bonds of organic molecules, eventually combine to form water in which the electrons are at their lowest level of potential energy, as far as protoplasm is concerned. The energy released in this process is used by protoplasm for its maintenance and for making more of itself from the materials of its environment. The oxidative process does not occur spontaneously but is catalyzed by the iron porphyrin or heme enzymes.

The reverse reaction is essentially the process of photosynthesis. Water is acted upon by sunlight in the presence of chlorophyll to produce electrons of high potential energy, plus O_2 , plus protons.

143. THE NATURE OF THE GOLGI APPARATUS. George E. Palade, The Rockefeller Institute for Medical Research. (Introduced by Robert Chambers.)

The various appearances and locations of the Golgi apparatus can be duplicated in vertebrate somatic cells by myelin figures that develop intracellularly after treatment with 40–55% ethanol. This obtains for all kinds of cells tested, including rat liver, kidney, pancreas, intestinal and epididymal epithelia, fibroblasts, endothelia and smooth muscle fibers; rabbit and chick nerve cells, and epithelia and muscle fibers from chicken gizzard. The various tissues were “homogenized” in saline or sucrose solutions and the cellular suspensions thus obtained treated thereafter with ethanol and sudan black.

The myelin figures developed at the expense of pre-existing refringent droplets, some of which stained supravitaly with basic dyes, especially with neutral red. According to their staining affinities, these inclusions are lipid and contain triglycerides and phospholipids in various proportions.

Similar intracellular myelin figures were produced by other means such as appropriate H^+ -concentrations ($pH=5.0-5.8$), prolonged or intense vital staining, and partial desiccation.

The current Golgi apparatus techniques were followed step by step on thin, transparent tissue “squashes” in order to find out if myelin figures are induced intracellularly during their application. It was found that the fixatives most commonly used for the demonstration of the apparatus, namely Nassonov, Aoyama, and daFano mixtures, actively promote the formation of intracellular myelin figures. After subsequent osmium impregnation such figures were identical to the classical Golgi apparatus.

It is concluded that the GA of somatic cells is an artifact, namely a collection of myelin figures induced intracellularly during fixation.

144. THE FORMATION OF CERTAIN CONNECTIVE TISSUE FIBERS. Parker Vanamee and Keith R. Porter, The Rockefeller Institute for Medical Research.

Arrays of connective tissue fibers were produced by growing explants of chick and rabbit embryo skin in tissue culture on plastic coated cover slips. These fiber mats were fixed over osmic acid vapors and mounted with the plastic membranes for electron microscopy.

The predominant type of fiber observed (unit fiber) has a diameter usually less than 500 Å. Such fibers are long slender strands, which, at their ends, may taper off gradually into fine beaded threads 50 Å or less in diameter. The unit fibers of smaller diameter show striae of uniform size which are evenly spaced at 270 Å. In the presumably more completely formed unit fibers the striae are compressed into polarized groups of three which have an over-all length of 650 to 800 Å. Strands visible with the light microscope consist of groups of unit fibers with their striae in in phase, usually having the characteristic periodicity of collagen (650 Å).

A second type of striated fiber up to 6μ in length and 300 to 400 m μ in width and tapered at both ends was occasionally seen among the collagen fibers. These fibers have a period measuring from 1,000 to 1,100 Å and are thought to represent a component of the connective tissue ground substance.

145. IDENTIFICATION OF "NEARO-CARCINOMA" CELLS IN THE HUMAN CERVIX USING A SURFACE-BIOPSY MODIFICATION OF THE PAPANICOLAOU TECHNIQUE. J. Ernest Ayre, M.D., Director, Cytological Laboratories, McGill University, Montreal. (Introduced by Secretary.)

The cellular material of the oral surface of the cervix can be obtained by making a circular scraping by means of a specially formed wooden spatula. The scrapings are smeared on to glass slides and stained for examination under the microscope. Such specimens are presented in the demonstration and if there are epithelial cells which are on the way to developing malignancy they can be distinguished from normal epithelial cells. By this means it is possible to diagnose a state of developing cancer far earlier than has hitherto been possible.

146. CONTRAST CONTROL IN PHASE MICROSCOPY FROM APPROPRIATE CHOICE OF MOUNTING MEDIUM. Oscar W. Richards, American Optical Co. Research Department, Buffalo, N. Y.

The contrast in the image seen with a phase microscope can be regulated by changing (1) the absorption (general or selective by wave length), (2) the optical path difference between the specimen and its surround, or (3) the width of the annulus in the phase system. The first two may either involve changes in the specimen or in the diffraction plate of the microscope objective, while the third involves the instrument only. Increasing the difference in refractive index of the mounting medium increases the contrast. With living specimens the control is limited to the use of nontoxic mounting media, but for other material a wider choice of media is available. Some media also transparentize the specimen as well as change the optical path difference. Others have different dispersions for light of different wave lengths so that color contrast may be combined with phase contrast. Photomicrographs and data will illustrate some of the possibilities of differential visualization of structure that may be obtained by the use of a suitable mounting medium.

147. TRIDIMENSIONAL CELLULAR STRUCTURE. Oscar W. Richards, American Optical Co. Research Department, Buffalo, N. Y.

Methods for the microscopic examination of the depth and thickness of a preparation include: sectioning and reconstruction, stereophotomicrography and interference microscopy. The first may be mechanical sectioning for which methods are established and optical sectioning has been discussed by Lucas (J. Franklin Inst. 1934, 217: 661). Now that phase microscopy makes possible examination of unstained living material, the use of stereophotomicrographs becomes possible. The more transparent images from the bright and the B- dark phase objectives is often more useful than from the denser A- dark contrast

objectives. The interference microscope reveals thickness as a function of optical path difference in the form of contours or may be used to examine the nature of reflecting surfaces. The methods and their limitations will be outlined and photomicrographs exhibited to show the potentialities of the latter two methods.

148. THE PROBLEM OF CHEMOAUTOTROPHY IN *CHILOMONAS PARAMECIUM*. W. B. Cosgrove, New York University and State University of Iowa. (Introduced by R. P. Hall.)

The Pringsheim strain of *Chilomonas paramecium* has been carried through three series of more than 20 transfers on the apparently inorganic media of Burrows and of Schoenborn in glass-covered culture flasks under increased CO₂ tension, and seems capable of continued transfer in these solutions. Maximum populations do not exceed 1000–2500 organisms/ml and vary with different batches of medium. There was no significant increase in nitrite or nitrate during growth, although thermodynamic calculations indicate that even with these populations significant amounts should be formed if *Chilomonas* oxidizes ammonia to either of the products. Attempts to increase the maximum population by varying the concentration of ammonium salts and by supplementing the media with vitamins, Fe⁺⁺, trace elements or nitrite were unsuccessful. The use of cotton plugs increased populations 10-fold. The slopes of the logarithmic portion of the growth curves in inorganic and in acetate media are identical; growth ceases much earlier in the inorganic medium. There is a simple linear relationship between the acetate content of the medium and the maximum population obtained. Carbon analyses of the inorganic salts and of the chilomonads show that the salts contribute enough organic material so that no increase in organic carbon following growth in inorganic media need be postulated. Analyses, in terms of further information now available, of the results of Mast and Pace on this problem indicate that their techniques were inadequate to demonstrate chemautotrophy. Burrows' results appear to be invalidated by the use of cotton plugs.

149. SYNTHESIS OF MEMBERS OF THE VITAMIN-B COMPLEX BY *CHILOMONAS PARAMECIUM*. George G. Holz, Jr., New York University and University of California at Los Angeles. (Introduced by R. P. Hall.)

The observations of Hutchens et al. ('41), that *Chilomonas paramecium* synthesizes nicotinic acid in an acetate and ammonium-N medium, have been confirmed by microbiological assays. In addition, synthesis of pyridoxal and riboflavin has been demonstrated by similar techniques.

Bacteria-free cultures of *C. paramecium* (Pringsheim strain) were used after 10–25 previous serial transfers in an acetate and ammonium-N medium (supplemented with thiamine) in Pyrex glassware (flasks covered with beakers). Cultures were centrifuged in the logarithmic growth phase and the concentrated flagellates submitted to the following extractions: methods of Krehl et al. ('43) and Johnson ('45) for nicotinic acid; method of Rabinowitz and Snell ('47) for pyridoxal; method of Strong ('47) for riboflavin. Vitamin concentrations of extracts were determined by microbiological assays: methods of Gaines and Stahly ('43) and Johnson ('45) for nicotinic acid; method of Rabinowitz et al.

('48) for pyridoxal; methods of Snell and Strong ('39) and Strong ('47) for riboflavin. Results were calculated and criteria of reliability applied according to recommendations of Snell ('47). Representative assay values are: nicotinic acid, 1.25 μgm (117.92×10^5 flagellates); pyridoxal, 0.40 μgm (229.15×10^5 flagellates); riboflavin, 0.63 μgm (473.1×10^5 flagellates). These values represent amounts of vitamins extracted and are not to be interpreted as absolute values in terms of number of organisms submitted to extraction.

Preliminary studies, still considered inconclusive, suggest that *C. paramecium* may also synthesize pantothenic acid but probably does not synthesize thiamine.

150. COMPARATIVE MORPHOLOGY OF CERTAIN CILIATES IN THE COLPIDIUM-GLAUCOMA-TETRAHYMENA GROUP. John O. Corliss, New York University. (Introduced by R. P. Hall.)

A comparative morphological study of the still controversial *Colpidium-Glaucoma-Tetrahymena* group of holotrichous ciliates is being made in an attempt to bring to light their true taxonomic interrelationships. Bacteria-free strains, which have been used in nutritional and other biochemical investigations, have been collected from various laboratories and maintained in both bacteria-free and bacterized cultures. Studies of living animals and material stained by relief methods (China blue, Opal blue, nigrosine) have been completed. Data so obtained (including number of ciliary meridians, size and position of cytostome, position of contractile vacuole, length and breadth of body) will be presented in charts and camera lucida drawings. In further work with silver impregnation, Feulgen reaction and haematoxylin stains, particular attention will be given to the nuclei. The investigated strains and species include: *Colpidium campylum* (strain isolated at Fordham University and used by Wilber, Seaman and others), *Tetrahymena geleii* E (Elliott's strain, formerly known as *Colpidium striatum*), *T. geleii* GP (referred to as *Glaucoma piriformis* by Hall and associates until 1945), *T. geleii* H (Hetherington's "*Colpidium campylum*" strain), *T. geleii* L-I (strain recently isolated by Loefer), *T. geleii* L-II (a second strain isolated by Loefer), *T. geleii* T-P (Thomas-Phelps strain, used by Furgason in 1940 in his erection of the genus), *T. geleii* W (used extensively by Kidder and associates) and *T. vorax* (the second recognized species of *Tetrahymena*).

151. STUDIES ON THE BIOTIC POTENTIAL OF DROSOPHILA. John M. Carpenter, University of Tennessee.

The biotic potential of any organism is a measure of its potential ability to reproduce. Such a measure involves the determination of significant figures for fecundity, viability, generation time and sex ratio. Determination of absolute values for these figures is extremely difficult but may be closely approached under rigidly controlled laboratory conditions using refined techniques.

Studies on the biotic potential of a strain of *Drosophila melanogaster* were made in the laboratory at a temperature of $23^{\circ}\text{C.} \pm 1$. Healthy adult males and females within one hour of eclosion were used. These were obtained from an inbred strain derived from a single female fertilized in nature. Ten females were isolated singly in shell vials with two males to insure insemination. Fresh food

on a paper slip was inserted into each vial every 24 hours for measurement of fecundity. Food used was of constant and high nutritive composition. Each food-slip, containing eggs laid by one female during 24 hours of oviposition, was placed in a bottle containing enough food for development of adult offspring. These offspring furnished data on viability, generation time and sex ratio. The entire experiment was repeated after an interval of several months.

Average time for a generation was 10.17 days, fecundity per female per generation 715.5, viability 0.697, and sex ratio 0.496. These figures are being analyzed statistically for significance.

The product of these figures represents the biotic potential of a *Drosophila melanogaster* strain during a generation. Such studies are believed of importance in the study of animal populations from the standpoint of evolution.

Demonstrations by Motion Pictures (Section C)

152. THE EFFECT OF LOCAL X-RAY IRRADIATION UPON THE DEVELOPMENT OF VARIOUS PARTS OF THE BODY OF THE YOUNG MOUSE. V. V. Brunst, Frank H. J. Figge, and D. J. Barnett, Departments of Anatomy and Radiology, University of Maryland Medical School. (14 min.)

The first part of the film demonstrates the method of local irradiation of two-day old mice of the C_{57} (black) strain.

The second part demonstrates the effect of local irradiation. In addition to the other effects, all irradiations resulted in temporary inhibition of hair development. After some time, growth of non-pigmented hair was observed. The irradiation of the middle part of the body in many cases, paralyzed the posterior limbs and the tail. Obviously, this is a result of partial inhibition of vertebral column growth which gives rise to compression of the spinal cord. Irradiation of the head inhibited the development of the treated region which caused a general disturbance of the animal's movements. Irradiation of the posterior limbs and the tail permanently inhibited the growth of treated parts.

153. PERIPHERAL VASCULAR REACTIONS INDUCED BY X-IRRADIATION OF A PORTION OF THE WING OF THE BAT, *MYOTIS LUCIFUGUS*. Douglas E. Smith, George Svihla and Harvey M. Patt, Argonne National Laboratory. (Introduced by Theodore N. Tahmisian.) (10 min.)

X-irradiation of a portion of the wing of the bat, *Myotis lucifugus*, induced a series of vascular reactions, the quality and quantity of which were related to the amount of irradiation administered. The smallest dose (124,000 r) caused leucocyte sticking in all types of vessels. Administration of 248,000 r was followed by stagnation of blood in many of the smaller vessels. Increasing the amount of irradiation to 1,240,000 r resulted in the sticking to vessel walls of large masses apparently composed of blood platelets. A more widespread stagnation of blood was also seen. The highest dose (4,960,000 r) was followed by complete flow failure. With the higher doses, failure of venomotion and a marked leakage of fluid from the vascular system were evident.

154. ARTIFICIAL INSEMINATION OF RABBITS AND TRANSPLANTATION OF RABBIT EGGS (MOTION PICTURE). M. C. Chang and G. Pincus, Worcester Foundation for Experimental Biology, and Tufts College of Medicine.

The pictures of Spallanzani (first successful artificial insemination of dogs in 1785), Heape (first successful transplantation of rabbit eggs in 1890), Hammond and Walton (who developed the technique of artificial insemination of rabbits), and Pincus (who developed the technique of transplantation of rabbit eggs) are shown. Methods of rabbit semen collection by means of a piece of rabbit skin and artificial insemination of the rabbit are photographed in color. The recovery of rabbit eggs from excised oviducts and from the living animal by a surgical method are demonstrated. Living rabbit eggs in the 4-cells stage are photographed. Surgical methods for transplanting rabbit eggs at early stages into the oviducts, and blastocysts into the uterus of recipient rabbits are shown. The storage of eggs at low temperatures and the probability of eggs (recovered at different stages, and stored at various temperatures for different lengths of time) developing into normal young rabbits are described. Albino young produced from black chinchilla foster mothers are photographed. A historical review of sperm study and the development of artificial insemination for livestock improvement are shown in a chart. The egg study and the possibility of egg transplantation for the full utilization of female germ cells of valuable animals as illustrated for livestock improvement.

155. NESTING HABITS OF THE DIAMOND-BACK TERRAPINS. Irwin C. Kitchin, University of Georgia. (13 min.)

The breeding pens at the Beaufort, N. C., station of the U. S. Fish and Wildlife Service furnished the opportunity to film the digging of the nest, egg-laying and the covering of the nest by the diamond-back terrapin. The reflex nature of nesting is illustrated by moving an actively nesting female, just after the eggs were deposited, to barren sand where she continues the typical movements of nest covering and camouflaging. The film also includes shots of the Beaufort station, the breeding pens and the feeding and culture methods employed.

156. AN AUTO-CHROMO-PHOTOMICROTOME. Erling S. Hegre, Medical College of Virginia. (15 min.)

A film showing the apparatus and illustrations of its application to the study of the minute anatomy of biologic specimens in serial section.

The apparatus described is designed to cut, stain and photograph serial levels through a specimen embedded in paraffin. By a special method of staining the details encountered at each level are recorded faithfully from the cut surface of the block, thus eliminating the distortions which inevitably occur during the preparation of serial sections by previous methods. The resulting film record is suitable for rapid (movie) projection and study.

To illustrate the application of this apparatus and technique to teaching and research the following scenes are presented:

1. Horizontal levels through an embryo at 2μ intervals. The levels are accurately superimposed and the relationship of the various structures can be observed clearly and with continuity.

2. Oriented horizontal levels through an embryo. In this scene the horizontal levels are viewed at an angle and are seen in their normal relationship to the remainder of the specimen.

3. Oriented sagittal levels through an embryo. In this scene the embryo is partially exposed and each level is seen against a background of the remainder of the specimen.

4. The mesonephric kidney; serial levels through the nephron taken at high magnification.

157. CINE-PHOTOMICROGRAPHS OF IMPLANTS OF TANTALUM IN FROG TADPOLES. Carl Caskey Speidel, University of Virginia.¹ (Motion picture; 15 min.)

These motion pictures were made directly from living frog tadpoles in which tantalum powder or wire had been implanted. They portray the principal cellular reactions induced by the implants over periods that vary from 5 hours to 80 days. Many of the scenes pictured were photographed at low speed and show all cell movements greatly accelerated.

(Cf., paper no. 93.)

¹ Aided by a grant from the American Cancer Society (Committee on Growth). The tantalum was supplied by the Ethicon Suture Laboratories, New Brunswick, N. J.

158. REPRODUCTIVE BEHAVIOR IN THE AFRICAN MOUTHBREEDING FISH, TILAPIA MACROCEPHALA (BLEEKER). Lester R. Aronson and A. Marie Holz-Tucker, The American Museum of Natural History. (30 min.)

Reproductive behavior in this Cichlid fish is illustrated by Kodachrome motion pictures. Among the early courtship patterns, throat-puffs, head-nods, and tail-slaps are most readily distinguished. In the group of behavioral acts that are more closely associated with the spawning are nest-building, passing-nest, and spawning-quivers. While all of these behavioral patterns are exhibited in a qualitatively similar manner by both the male and female, there are noticeable differences in the frequency of occurrence of each item of behavior for the two sexes. For several of the patterns a temporal fluctuation in frequency in relation to the time of oviposition is evident.

After the spawning, scenes show (1) the eggs being picked up by the male; (2) eggs and later embryos rolling around within the male's mouth; (3) the male's modified reaction to food, and (4) the newly released fry.

The time of ovulation is estimated from the frequency of some of the behavioral acts, and this made possible pictures showing the differences in external appearance of the ripe unovulated ovary, the ovulated ovary before oviposition, and the spent ovary, as well as a highly magnified view of the actual process of ovulation in vitro.

159. THE BURYING BEETLES. Lorus Milne and Majorie Milne, University of New Hampshire. (8 min.)
160. SPERMATOOA OF THE VERTEBRATES. Edmond J. Farris, The Wistar Institute. (15 min.)

General Physiology (Section A)

161. THE EFFECT OF LOW ENVIRONMENTAL ATMOSPHERIC PRESSURES ON THE CHOLESTEROL CONTENT OF THE BLOOD IN THE PIGEON. Louis A. Susca, The New York Medical College, Flower and Fifth Avenue Hospitals. (Introduced by Secretary.) (10 min.)

Susca and Wilber (1949) reported changes in the cholesterol, glucose and phospholipid content of the blood of turtles which were exposed to low environmental atmospheric pressures. Studies of the cholesterol content of the blood of pigeons exposed to low environmental atmospheric pressures have also been completed. Cholesterol estimations according to the method of Bloor (1916) were made on the blood of each of ten specimens of pigeons of the variety *Columba* sp.; these were exposed to pressures equivalent to altitudes of 18,000; 33,000; 48,000; 64,000; and 90,000 feet, respectively. Cholesterol estimations were made also on the blood of untreated pigeons. Mean values (of 10 pigeons) for blood cholesterol, in milligrams per 100 cc of blood, in experimental and control pigeons, are as follows: controls, 82.8; 18,000 feet, 125.5; 33,000 feet, 150.1; 48,000 feet, 88.3; 64,000 feet, 119.9; and 90,000 feet, 64.8. It is evident that there is an increase in blood cholesterol in pigeons up to and including exposures to 33,000 feet simulated altitude. At higher altitudes, there is an irregular decrease in the amount of cholesterol in the blood of the pigeons. A further, complete report of the effects of low environmental atmospheric pressures on the cholesterol content of the blood of various vertebrates, is now in progress.

162. RESPIRATORY MECHANISM IN THE RAT. F. H. McCutcheon, University of Pennsylvania. (Lantern; 15 min.)

Manometric and volumetric analyses of breathing cycles in the rat show an intra-pulmonary retention and compression of a portion of inspired air.

An intra-alveolar gas pressure (more than + 20 mm Hg), significant for respiratory gradients, is indicated by calculations based on correlated pressure and volume data. Direct measurements of intra-alveolar pressure have not been made.

The evidence supports a hypothesis that terminal bronchiolar and other intra-pulmonary myo-elastic tissue actively regulates intra-alveolar pressure.

163. HISTOPATHOLOGY OF X-RAYED LARVAL URODELE TISSUES, EXPOSED AND MAINTAINED AT DIFFERENT TEMPERATURES. John P. O'Brien, Marquette University. (8 min.)

The advantage of using a poikilothermous organism in studies directed to the problem of radiosensitivity in relation to metabolic rate will be clear. Externally observable responses of x-rayed larval *Amblystoma punctatum*, 15-20 mm, exposed

and maintained at different temperatures, together with the details of procedure, were previously reported (O'Brien, '47, Anat. Rec. 99, 4).

This report concerns a histological analysis of some 50 of these larvae. The study has, thus far, been confined to the stratified epithelial tissues of the intergumentary and digestive systems. Epithelial elements are generally regarded as among the more radiosensitive tissues, and their relative simplicity and uniformity would appear to enhance their value in the assaying (quantitative) of cellular damage. The material was fixed in Bouin's, sectioned at 5μ and stained with Delafield's haematoxylin and eosin.

Damage to the epithelia is manifested principally in (1) a variety of chromatin disarrangements, (2) varying degrees of cytoplasmic, nuclear and whole-tissue degeneration. The observations reveal that the epithelia of larvae maintained at 12° – 15° C., following exposure to 3136 r at 7° – 9° C. or 28° – 26° C., suffered less damage than those maintained at 22° C.– 24° C. It is not evident from this study that the temperature at the time of exposure is important in the sum-total damage effected by radiation; it would, however, be definitely premature to judge that it is *not* a significant factor. Clearly, what is needed is material offering a less equivocal and more delicate criterion of damage, preferably a *functional* criterion, such as growth activity or respiratory exchanges. Such experiments are planned.

164. INDIRECT EFFECTS OF X-RADIATION ON THE WALL OF THE DEVELOPING MESENCEPHALON OF THE CHICK EMBRYO. M. J. Tobin and J. P. O'Brien, Marquette University. (7 min.)

The 72-hour chick mesencephalon is composed of (1) mitotically active *germinal* cells, ependymal layer, adjacent to the lumen, (2) mitotically inactive *epithelial* cells, mantle layer, augmented by cells from ependymal layer. These features recommend this tissue for the study of indirect effects of x-radiation.

Ninety-nine 72-hour embryos, *in ovo*, constituted groups: (A) controls, (B) totally x-rayed eggs, (C) embryos alone protected from x-radiation, (D) embryos alone x-rayed. Protection of parts was effected by lead plating of appropriate shape and thickness. Radiation conditions: 140 kv., 15 ma., no filter added, target-shell distance 23 cm, 300 r/min; dosage 600 r. Embryos from each group were fixed for sectioning following 24 and 48 additional hours incubation.

Changes, group B, 96 hours: brain wall thinner than in controls; hemorrhagic areas; cellular disintegration, manifested principally in germinal cells in terms of nuclear debris, reduced mitotic activity, abnormal mitoses, vacuolization, pyknosis; ependymal and mantle layers hardly distinguishable. At 120 hours a remarkable recovery from the radiation damage is manifested in this group. Changes, group C; little alteration detectable at 96 hours; at 120 hours, however, distinct pathological changes are apparent, particularly in the *germinal* cells; these changes are less severe and more delayed than in group B, but they appear to be qualitatively similar. Changes, group D: these are rather similar to those in group B; there are, however, definite suggestions that the damage is lesser, as would be expected on the basis of the results in group C.

These results clearly demonstrate histologically detectable *indirect* effects of x-radiation; these would undoubtedly be augmented with the application of larger doses of radiation.

165. FURTHER INVESTIGATION OF THE INFLUENCE OF LOCAL X-RAY IRRADIATION ON THE TAIL DEVELOPMENT OF THE YOUNG AXOLOTL, *SIREDON MEXICANUM*. V. V. Brunst, E. A. Sheremetieva-Brunst, Frank H. J. Figge, and D. J. Barnett, Departments of Anatomy and Radiology, University of Maryland Medical School. (14 min.)

The first sign of irradiation effect (inhibition of growth and changes in pigmentation) was noted 23 days after irradiation with large doses. Histological examination showed a clear picture of the degeneration of pigment cells. The first giant cells (macrophages or polyblastes) were noted 34 days after irradiation. This is the beginning of the primary acute reaction of irradiation.

This investigation showed that not all elements of our general schema of acute primary reaction of treated organs to irradiation are equally important and permanent. In all cases, after an extended post irradiation period, only a degenerating pigment layer and giant cells (macrophages or polyblastes) were observed in irradiated tissues. The accumulation of small cells in the "zone of stimulation" was observed only in some cases of inflammatory reaction. Obviously the time of formation of this zone is very short. However, it was possible to observe this phenomenon if the animal was fixed during this period of stimulation. Degenerating giant epithelium which is generally very typical of the primary acute reaction to irradiations (which was described in 5 of our previous papers) was not observed in this material. This showed that the reaction to irradiation is not always observed in the same form.

This investigation showed that the beginning of the primary acute reaction and the secondary stable state appear within the same period of time after irradiation that had been observed previously.

166. THE UPTAKE OF RADIOPHOSPHORUS BY VACCINIA VIRUS AND ITS ASSOCIATED COMPONENTS. Luolin S. Altenburg, The Rice Institute. (Introduced by H. J. Muller.) (15 min.)

Since phosphorus is a component of nucleic acid, a substance common to all viruses, a study of the incorporation of its radioactive isotope into the virus particle has been undertaken in the hope that a general method for detecting all viruses grown on hens' eggs could be worked out. A further object has been to develop a sensitive method for the study of the virus growth.

Radiophosphorus was injected into the albumen of developing eggs and the vaccinia virus grown on the chorio-allantoic membrane. A greater amount of undialyzable radiophosphorus was then found in the allantoic fluid of inoculated eggs than in that of uninoculated. However, it was necessary to make counts from a relatively large number of eggs to establish a significant difference. Therefore measurements of radiophosphorus of the infected membranes themselves were made, after the membranes were weighed, macerated, centrifuged, and the supernatants dialyzed. When the virus was allowed to grow 72 hours, no significant difference was observed between inoculated and uninoculated eggs. But when the virus growth periods were shortened to from one to three hours, a difference existed between the inoculated and uninoculated portions of the same membranes,

as well as between the inoculated portions of these membranes and membranes from uninoculated eggs, with the greatest difference existing at the 1½-hour growth period, at which time also the level of phosphorus activity reached a peak, being roughly three times as high as at the 1-hour period and falling off to about the same level by the end of the second hour.

167. THE EFFECT OF X-RADIATION ON THE METABOLIC PROCESSES OF THE RESTING CELL, Theodore N. Tahmisian, Argonne National Laboratory. (Lantern; 15 min.)

X-radiation dosages ranging from 10,000 r to 200,000 r do not completely inhibit respiration in the diapause grasshopper embryo (*Melanoplus differentialis*). Respiratory, morphological, and cytological studies revealed that anabolic enzymes are associated with the nuclear material and are more susceptible to X-irradiation damage than the catabolic enzymes. In a resting cell a nucleus is necessary for maintenance of the status quo of the cell. Following X-irradiation a resting nucleus does not have to undergo mitosis to become pyknotic. Pyknosis of the resting nucleus depends upon the X-ray dosage, upon the state of metabolism, and upon time. Repression of irradiation damage to the cell can be obtained by subjecting the cells to submetabolic temperatures. The integrity of the nuclear chromatin material is dependent upon its functional potency.

168. EFFECTS OF X-RAYS ON I^{131} UPTAKE IN RATS AND MICE. Titus C. Evans and Estelle S. Sobel, The State University of Iowa. (Lantern; 10 min.)

Contrary to our findings with rats, mice (Swiss strain) have exhibited a very low uptake of I^{131} in the thyroid and such uptake has not been increased following total body exposure to roentgen radiation.

Our previous finding that total body irradiation increased the uptake of iodine in the thyroid during the week following exposure has been confirmed by additional experiments on rats of another strain and on the same diet as were the mice. This increased uptake appears not to be an effect on the thyroid directly. It is apparently a reflection of tissue damage which releases some agent having properties such as to cause the temporary increase in iodine uptake. This is supported also by recent findings in the literature that local irradiation of the thyroid did not increase the rate of oxygen consumption of rats, whereas total body exposures have been found by other investigators to result in a temporary increase in metabolic rate.

All of the possible explanations for the failure of mice to respond in the same way as the rats have not yet been explored. A wide range of exposures have been used. These will be applied to different areas of the body and the effects of a low iodine diet on the iodine¹³¹ uptake with and without total body irradiation will be studied in the near future.

169. EFFECTS OF X-RAYS ON THE MITOTIC STAGES IN THE EPIDERMIS OF THE NEW-BORN MOUSE. Estelle S. Sobel, The State University of Iowa. (Introduced by Titus C. Evans.) (Lantern; 5 min.)

It has been found in preliminary experiments that the usual ratio of one phase to the others is altered within an hour following exposure to low voltage x-rays. Results have been obtained at an exposure of 1900 r at 15 minutes, 30 minutes, 45 minutes, and 60 minutes following exposure. The most definite effect observed, which progressed with the time after irradiation, was a reduction in the percentage of telophases.

In additional experiments, exposures of 1900 r, 1300 r, 900 r, and 47 r were used and the tissues examined at $1\frac{1}{2}$ hours. Some alteration in the frequency of telophase was found in the epidermis exposed to dosages as low as 47 r.

170. RETICULOCYTOSIS FOLLOWING INJECTION OF ANOXIC PLASMA IN DOGS.¹ F. W. Kinard and Daniel W. Ellis, Medical College of the State of South Carolina. (10 min.)

Normal rabbits, injected intraperitoneally with plasma from rabbits which had been exposed to low atmospheric pressure for 4 hours, in most cases showed an erythrocytosis and in some an increased hemoglobin. The increase on the first and third days were considered to border on significance (Bonsdorff and Jalvisto, '48). Bonsdorff ('48) observed an erythrocytosis of 0.5 to 0.8 million and a reticulocytosis of 6-7% in normal rabbits following intraperitoneal injection of 3 cc of plasma from normal rabbit's blood which had been exposed to an atmospheric pressure of 200-400 mm Hg for 4 hours. It was postulated that erythropoietic substances appear in the plasma when whole blood is exposed in vitro to low atmospheric pressure.

Ten normal dogs were injected intraperitoneally with 10-11 cc/kg, and 6 dogs were injected intravenously with 1-5 cc/kg of plasma prepared from heparinized dog's blood which had been evacuated at 200 mm for 4 hours. Hematocrit, hemoglobin, and reticulocyte studies failed to give unequivocal evidence of bone marrow stimulation. It is planned to use a larger series of animals for further study.

¹ This research was aided by a grant from the American Association for the Advancement of Science.

171. INDEPENDENT OCCURRENCE OF AUDIOGENIC SEIZURES AND MIDDLE-EAR INFECTIONS IN INBRED MICE. Dorothea Starbuck Miller and Mildred J. Zamis, The College, University of Chicago. (Lantern; 10 min.)

In view of recent reports that audiogenic seizures in rats are associated with middle-ear infections, it seemed advisable to make a histological examination of the ears of our inbred strains of mice which are susceptible (dba) and non-

susceptible (C57 black) to sound-induced convulsions. Serial sections of the ear region were made from animals of each strain which suffered non-fatal and fatal convulsions, and of those which failed to convulse.

In the dba (seizure-susceptible) strain, the following results were obtained. Of 17 animals which failed to convulse, two showed heavy infections of both middle ears, one had a light infection in one ear, and in 14 cases the ears were clear. One animal which convulsed and recovered showed no infection. Of 7 mice which suffered fatal convulsions, 6 had perfectly clear middle ears, while one showed a heavy infection. In the dba strain, the incidence of middle-ear infections is low; of 25 animals examined, only three showed massive infections and one a light infection. This condition appears to be unrelated to audiogenic seizures.

In the resistant strain (C57 black), 12 mice were examined. Among 7 which failed to convulse, 6 showed clear middle ears, while one had a light infection. Of 4 which convulsed and recovered, two were not infected, one showed a massive infection of both ears, and one a light infection. One C57 which convulsed and died showed a heavy infection of both ears. A larger group of C57 animals is being prepared and additional results will be presented.

172. A SUGGESTED MECHANISM OF CONVULSIVE SEIZURES, DERIVED FROM OBSERVATIONS UPON PERIPHERAL NERVE. J. E. P. Toman, M. D. Greenhalgh and L. M. Henrie, University of Utah College of Medicine, Salt Lake City. (15 min.)

Prolonged changes in excitability following repetitive electrical stimulation of frog sciatic nerve have been examined as functions of frequency, pulse duration, polarity, voltage, and total duration of the stimulus, and as modified by a variety of drugs and by alterations in temperature. With slightly supraliminal shocks there is usually a small increase in threshold lasting several seconds in the responding fibers. This effect is overshadowed by a facilitation of the nonresponding fibers lasting for several seconds when the majority of fibers is activated by submaximal shocks. With supramaximal stimulation of several times the rheobasic voltage there occurs a progressive fall in threshold to 50% or less of the control value, followed by an exponential recovery lasting many minutes and having a half-time of approximately one minute at 28°C. The decrease in threshold is associated with a slight decrease in amplitude, a loss of the super-normal phase of recovery, and an increase in synchronization of repetitive discharges, but these do not recover at the same rate, suggesting a shift in the relative fractions of the membrane potential. Continuous stimulation throughout the period of facilitation eventually forces the threshold above control values, and recovery is then greatly prolonged. The time characteristics of this phenomenon, its alterations with changes in temperature and stimulus parameters, and its prevention or reversal by anticonvulsant drugs in low concentration, all suggest a possible role of supramaximal facilitation in the initiation, spread, and ultimate self-limitation of electrically induced convulsions in the central nervous system.

173. THE ELECTRICAL EFFECTS OF TOLSEROL (MYANESIN). R. Beutner and T. C. Barnes, Hahnemann Medical College and Hospital of Philadelphia and The Keeley Institute, Dwight, Ill. (Lantern; 8 min.)

The successful use of tolserol for alcoholic patients at the Keeley Institute suggested that bioelectrical studies on animals might throw light on the mechanism by which tolserol abolishes the tremor and anxiety of chronic alcoholics (J. A. M. A. 140: 672, 1949). We used a 6-channel Rahm electroencephalograph recording the electroencephalogram, electromyogram, electrocardiogram, respiration and general spontaneous movements of the frog before and after injection of tolserol; 250 mg per kilogram (5–10 per frog) produced a generalized hypo-potentia within one minute reducing the voltage of action currents from muscle, heart and brain. The respiration decreased from 90 to 18 per minute, the pulse from 80 to 70 per minute, and the E.E.G. from 15 per second 20 microvolts to 9 per second 10 microvolts. In some animals the E.E.G. frequency declined to 3 per second (analogous to the sleep waves produced by tolserol—see Berger, J. Pharmacol. 96: 255, 1949). With dosage of 250 and 100 mg per kilogram the electrical effects of tolserol were usually reversible. The depression of the electroencephalogram was less pronounced than other effects. Gammon observed the disappearance of spike-and-dome waves in the electroencephalogram of petit mal patients after tolserol. The site of action is probably subcortical. According to McCulloch (Second International Congress of Electroencephalography, Paris, 1949) tolserol depresses the internuncial neurones, thereby inhibiting the regenerative circuits or neural “feed back” of impulses believed to be the basis of neurosis (according to the cybernetic theory of Norbert Wiener).

174. ANIMAL EXPERIMENTS SUPPORTING THE ELECTRICAL THEORY OF DRUG ACTION. T. C. Barnes and R. Beutner, Hahnemann Medical College and Hospital of Philadelphia. (Lantern; 15 min.)

Both the leech muscle and the oil-cell (for measuring phase-boundary potentials) have approximately the same limit of accuracy for detecting acetylcholine—about 1 part in 300 million. Deteriorated solutions of eserine lose both electrical and pharmacological activity at the same time; 0.5% eserine, freshly prepared, produced 30 millivolts negativity at saline-guaiacol interface but after ageing (purple color) electrical activity was lost. The potency of curare solutions can be assayed by the electrical method. Tubocurarine Squibb diluted $4 \times$ (0.65 mg per cubic centimeter) was found to have a potency of three head-drops units per kilogram per cubic centimeter tested on rabbits and produced a potential of 40 millivolts negativity at a saline-oleate-nitrobenzene interface. Tubocurarine diluted $10 \times$ (0.27 mg per cubic centimeter) contained one head-drop dose per kilogram per cubic centimeter and gave 20 millivolts. Intocostrin diluted $20 \times$ (1 unit per cubic centimeter) had a head-drop unit per cubic centimeter per kilogram and produced 10 millivolts. Aged deteriorated curare solutions lose electrical activity. The resistance of oil reduced from 2.4 megohms to 0.8 megohm by penetration of 0.002 M choline and from 2.4 to 0.27 megohms by acetylcholine. The greater ionization and electrogenic properties of acetylcholine ex-

plain its greater pharmacological action. The so-called "prosthetic groups" of Pfeiffer are probably factors influencing ionization and phase-boundary potential of drugs.

Endocrinology (Section B)

175. DEVELOPMENT OF THE BACULUM OR OS PENIS IN THE LONG-TAILED WEASEL.¹
Philip L. Wright, Montana State University. (Lantern; 10 min.)

The bacula of the male long-tailed weasel are of two types, immature and adult. They may be distinguished by appearance and weight. The immature baculum develops into that of the adult at the time sexual maturity is reached when the animal is about one year old. Immature weasels were castrated and when autopsied several months after the usual time for assumption of sexual maturity still showed the immature type baculum. Adult weasels were castrated and after several months no regression of the baculum was found. Immature weasels implanted with pellets of free testosterone exhibited at autopsy bacula intermediate between the adult and immature types. Another series of immature weasels was castrated and implanted with pellets of testosterone propionate. When autopsied after two months the bacula were essentially of the adult type in both appearance and weight.

¹ Aided by a Grant-in-Aid from the Sigma Xi Research Fund.

176. CHANGES IN THE THYROID GLAND OF HAMSTER EMBRYOS HYPOPHYSECTOMIZED BY DECAPITATION. Charles L. Foote and Florence M. Foote, Southern Illinois University. (Lantern; 15 min.)

Thyroid glands of hamster embryos, hypophysectomized by decapitation on the 12th day of gestation and allowed to continue development until the 16th day, are compared with glands of normal embryos. The technique of decapitation will be described.

Of 141 embryos decapitated only 23 were recovered alive, an 83.7% mortality. Comparative studies were made of glands of the decapitated embryos (Group H), 7 unoperated embryos recovered at the time of operation (Group A), and 11 intact embryos recovered when the mother was sacrificed (Group B).

In comparing average length of glands, Group B showed 79.3% increase over Group A, while Group H showed only 27.6% increase in length. However, a marked difference is indicated by the average total volume of the glands. Where Group B showed 202% increase over Group A, Group H showed a 17.8% decrease, making a difference in total volume between Groups B and H of 287.7%. This great difference between Groups B and H may be explained, in part, by the number and size of follicles, and the number of interfollicular cells. No follicles showed colloid, but lumens were present in most glands. Follicles of glands of animals of Group H were larger than those of Group B, although the glands of Group B contained an average of 41.1% more follicles, and also a greater number of interfollicular cells.

When the pituitary gland is removed by decapitation in hamster embryos there is little change in development of individual follicles, but there is a marked decrease in volume of the thyroid gland due to the formation of fewer follicles and a decreased number of interfollicular cells.

177. SPONTANEOUS RUPTURE OF THE VAGINAL CLOSURE MEMBRANE DURING PREGNANCY IN THE GUINEA PIG. William C. Young and Richard C. Webster, University of Kansas. (Lantern; 10 min.)

In 58 of 71 animals observed daily from the 15th day after mating until the termination of pregnancy spontaneous rupture of the vaginal membrane was seen. Macroscopically the gestational opening in the pregnant female differs from the estrous opening in the non-pregnant female: The membrane does not disappear completely, the edges appear to be broken rather than dissolved and the vaginal mucosa is more conspicuously hyperemic. In 46 of the 58 animals rupture occurred between the 22nd and the 28th days of pregnancy, therefore about the beginning of the second trimester. In no instance did rupture first occur after the 40th day. The final closure was most common between the 29th and 48th day which approximates closely the span of the second trimester (24th to 46th day). The third trimester until the day of parturition is for the most part one of membrane persistence, although in 4 females which gave birth to living young, opening was intermittent until parturition.

Since evidence from several sources indicates that there is a partial shift in the control of pregnancy from the ovary to the placenta about the 25th day, it is assumed that rupture of the membrane during the second trimester is indicative of an adjustment having a hormonal basis. Efforts to find correlation between rupture of the membrane and duration of the opening with litter size and the course of gestation that might be suggestive of the nature of this adjustment have thus far been unsuccessful.

178. EFFECT OF BLOOD SERUM FROM HYPOPHYSECTOMIZED FROGS, *RANA PIPIENS*, ON OVULATION IN VITRO. Paul A. Wright, University of Michigan. (15 min.)

In 1946 the author showed that the dosage of pituitary material which produced complete ovulation in recently hypophysectomized frogs was considerably less than the amount required for 100% ovulation in normal frogs. The present work was undertaken to investigate the hypothesis that the more sensitive condition of hypophysectomized animals could be explained at least in part by the release of a sensitizing hormone into the blood during the process of hypophysectomy. Blood serum was withdrawn from adult females at various times after hypophysectomy and its ability to sensitize or maintain frog ovary in vitro was tested. Blood serum from normal frogs and a preparation of FSH were also used for comparative purposes. The procedure consisted of placing fragments of normal ovary in vials each containing 10 cc Ringer's solution plus 0.02 cc blood serum and removing them after 6 to 7 hours to dilute pituitary solution where the progress of ovulation was observed.

Ringer's solution containing FSH or serum withdrawn two hours or 18 hours after hypophysectomy was found to maintain (and in some cases increase) the ovulatory capacity of ovarian tissue, while the responsiveness of similar fragments kept in Ringer's solution alone or in Ringer plus serum of normal animals diminished. Blood serum obtained 96 hours or as much as 9 days after hypophysectomy was ineffective. It is postulated that blood serum for a brief time following hypophysectomy contains an added quantity of FSH or hormone of similar action.

179. DEVELOPMENT OF THE DIMORPHIC PIGMENT SPOT OF THE SYRIAN HAMSTER. Ruth E. Shrader, Yale University, Department of Anatomy. (Introduced by Carroll A. Pfeiffer.) (Lantern; 15 min.)

The pigmented dorsolumbar skin spot of the Syrian hamster is characterized in the adult by the presence of androgen responsive intradermal pigment cells clumped around greatly enlarged sebaceous glands, and also by the presence of exceptionally coarse black hairs in addition to the customary yellowish coat hair. The structures peculiar to the adult region are not present at birth. Pigment is first seen grossly in the dimorphic pigment spot at from 10 to 12 days, and is associated with the appearance of heavy concentrations of black pigment in the hair roots. The sebaceous glands and intradermal pigment cells characteristic of the region are present at this age. The pigment continues to increase until approximately 14 to 16 days and then begins to disappear. This disappearance is accounted for by the movement of the pigment from the hair follicles into the hair shafts. By 24 days the area is grossly unpigmented. Both hair root pigment and dermal dendritic cell pigment are lacking. The sebaceous glands are well developed and are the predominant structure in the region. At 28 to 32 days there is a gross reappearance of pigmentation in the areas as the result of the development of large concentrations of intradermal dendritic cells. This represents the initial stage of the adult type of organization in this region. Testosterone propionate does not significantly hasten the initial appearance of pigmentation but intensifies pigmentation once it is present and prevents its disappearance which normally occurs at 24 days.

180. ANDROGEN PRODUCTION IN THE FEMALE BROWN LEGHORN FOWL. Elsie Taber, Medical College of the State of South Carolina. (Lantern; 15 min.)

Previous experiments have shown that precocious androgen production, measured by comb growth, in gonadotrophin-treated chicks was correlated with changes in the interstitial cells of the ovarian medulla. In chicks with relatively little comb growth, these cells were filled with osmic-stained droplets; in chicks with accelerated comb growth the cytoplasm of these cells was granular.

If granular interstitial cells are a major source of androgen, one should be able to observe the reappearance of lipid-filled cells in the hypertrophied ovaries of chicks following the withdrawal of exogenous gonadotrophin stimulation, and in the ovaries of older birds following the inhibition of endogenous gonadotrophin production.

Thirteen chicks received daily injections of 100 I.U. of PMS for 10-16 days and were killed 6-7 days after the final injection. Regression of the initially stimulated combs was noted after injections ceased. Ovaries of these birds were hypertrophied, but contained vacuolar interstitial cells, similar to uninjected controls. Controls injected throughout the period showed continued comb growth and granular interstitial cells.

Four 2-3-month-old birds received daily injections of 8 R.U. of alpha-estradiol-benzoate/100 gm body weight for 12 days. Retardation of comb growth was observed within 4 days. Ovaries of injected birds contained more osmically stained, vacuolar interstitial cells than those from untreated controls, which showed uninterrupted comb growth.

These results supply additional evidence that the interstitial cells in the medullary portion of the ovary may be a major source of androgen in the chick.

181. RESISTANCE TO COLD AND ANOXIA BY YOUNG RATS. H. A. Salhanick and J. L. Starr, Harvard University. (Introduced by Frederick L. Hisaw.) (Lantern; 15 min.)

When new-born rats are exposed to low temperatures (3°C .), they pass into a state of torpor characterized by a complete cessation of reflexes, a rapid decrease in body temperature, a cessation of cardio-vascular activity and discontinuance of respiratory movements. The animals show the purplish coloration of cyanosis and oxygen uptake, as determined by a modified Scholander-Edwards respirometer, is near zero. One and two day old animals in this condition can survive after exposure for about 9 hours. The length of exposure that can be endured decreases rapidly during the first 8 days of life and between the ages of 8 and 12 days the animals survive only about 4 hours. As the homeothermic mechanism begins to develop after the 12th day, there is a slight increase in ability to withstand low temperatures and on the 20th day, there is a marked increase in this respect. Animals above 10 days of age do not pass into torpor and survive only a short time after the rectal temperature has decreased below 6°C .

Resistance to anoxia was found to parallel the ability to withstand cold until the development of the homeothermic system began. It is concluded that the ability to withstand anoxia is a crucial factor in the ability of the animal to resist prolonged exposure to low temperatures.

182. LUTEAL FUNCTION IN THE IMMATURE RAT. C. T. G. King, M. Macauley, F. L. Hisaw, and A. B. Dawson, Harvard University. (Introduced by Leigh Hadley.) (Lantern; 15 min.)

Ovulation, and subsequent formation of physiologically active corpora lutea, were obtained in 26 day old rats by injection of 15 I.U. of PMS followed 56 hours later by 20 I.U. of PU. The corpora lutea thus obtained remain functional for 10 to 12 days as judged by vaginal smears and decidual reactions. Cholesterol is not present until the third or 4th post-ovulatory day, and thereafter it may vary from a complete negative to a strong positive whether or not

progesterone is present in the blood. Blood appears in the vagina on the third or 4th day after traumatization of the uterus due to fusion and rupture of sinusoids in the deciduomata. The decidual tissue undergoes rapid degeneration on or about the 12th day of luteal life. During the period of luteal life the interstitial tissue maintains a strong Schultz reaction. Just prior to the first normal ovulation it becomes regionally or totally negative, and after ovulation again rises to a high level. Hypophysectomy performed on the 4th to 7th post-ovulatory day results in (1) complete depletion of the luteal cholesterol, (2) absence of progesterone in the blood, and (3) a rapid degeneration of the decidual tissue. Hypophysectomized animals treated with prolactin maintain progesterone secretion, and in general, the cholesterol content of the corpora lutea is less than in non-hypophysectomized animals.

183. THE EFFECT OF PHYSIOLOGICAL STRESS ON WATER DIURESIS.¹ James H. Birnie and W. J. Eversole, Syracuse University. (Introduced by Robert Gaunt.) (Lantern; 15 min.)

The diuretic response elicited by stress producing stimuli (studied by Browne and co-workers) has been recognized as a feature of the adaptation syndrome. An attempt has been made to correlate the effects of stress on the rate of urine flow with adrenal cortical function.

Intact rats were subjected to such stress producing procedures as partial hepatectomy, CCl₄ treatment, operative trauma, exposure to cold, intraperitoneal injections of glucose, and the administration of a crude and somewhat irritating whole liver extract. Under the influence of such stimuli there was observed a consistent augmentation in the diuretic response to a single dose of water (4 cc/100 sq cm body surface) when the animals were tested 24 hours after application of the stress. Continuation of CCl₄ treatment beyond the second day did not further intensify the augmented diuresis. Increased water excretion was not seen in CCl₄-treated or partially hepatectomized animals which had been adrenalectomized for 18 hours. Inhibition of the expected diuresis by adrenalectomy suggests that the adrenal glands play a role in producing this renal response. The observation that 4 cc of cortical extract (Upjohn) administered with a single dose of water causes an increased urine flow in the intact normal animal is further suggestive evidence regarding the relation of adrenal cortical hormone to the augmented renal response.

In contrast to the above, rats subjected to cold and tested one hour after exposure showed a marked antidiuretic response. Animals tested one hour after receiving injections of whole liver extract likewise showed a marked decrease in the rate of urine flow. The reversal of the renal response elicited by the administration of the same agent at different time intervals is interpreted as an example of the physiological reversal encountered in passing from the shock to the counter-shock phase of the adaptation syndrome.

¹Aided by grants from the National Heart Institute and the Markle Foundation.

184. RESPONSE OF RAT SEMINAL VESICLES TO TESTOSTERONE PROPIONATE SUSPENSION IN METHYL CELLULOSE AND TESTOSTERONE ADSORBED ON ALUMINUM PHOSPHATE.¹ Harold Schraer and W. J. Eversole, Syracuse University. (10 min.)

Young male rats weighing approximately 65 gm were castrated and two weeks later were injected with 5 mg either of testosterone propionate macrocrystals suspended in methyl cellulose or testosterone adsorbed on aluminum phosphate. Groups of animals were sacrificed at intervals of 4 days following injection and their seminal vesicles removed and weighed on a torsion balance. Four days after injection of both types of preparations, the seminal vesicles averaged approximately 70 mg. This is essentially the same response obtained with testosterone propionate in oil but contrasts with the untreated castrate weight of 10 mg. Eight days after injection the macrocrystalline suspension and the absorbed material were equally effective in causing a further increase in seminal vesicle weight but stimulating effects of testosterone propionate in oil were no longer detectable. At the 12th and 16th days the seminal vesicle weights of the rats treated with macrocrystalline testosterone propionate had increased to 500 mg whereas these sex organs had decreased (to 180 mg) in rats treated with testosterone aluminum phosphate. The effects of the latter had completely subsided by the 20th day and the effects of the macrocrystalline suspension had ceased by the 28th day.

It is apparent, therefore, that both testosterone propionate macrocrystals suspended in methyl cellulose and testosterone adsorbed on aluminum phosphate were more effective in increasing seminal vesicle weights of castrate rats than was testosterone propionate dissolved in oil.

¹ Aided by a grant from Ciba Pharmaceutical Products, Inc.

185. DISSOCIATION OF METABOLIC AND MORPHOGENETIC THYROID FUNCTIONS IN BROWN LEGHORN FOWL. Beatrice Mintz and L. V. Domm, Whitman Laboratory of Experimental Zoology and Hull Anatomical Laboratory, The University of Chicago. (Lantern; 12 min.)

Administration of two drugs was employed with the expectation of stimulating separable effects of thyroid hormone on any of the characters classically observed. Dinitrophenol (a "metabolic stimulant"; see Fleischmann review, '47) was fed or injected into two capons at 10 mg/d. Acetylthyroxin (a "morphogenetic stimulant") was injected on alternate days into three roosters at 0.05-0.15 mg, and at 0.05-0.2 mg into 4 hypothyroidic males also fed 0.5-0.6 gm thiouracil daily. Controls were limited to three roosters (one on thiouracil) as data were available for comparable birds previously maintained in this laboratory.

Neither compound influenced comb growth or body weight. Dinitrophenol had no effect on feathers. Acetylthyroxin similarly failed to change plumage in otherwise normal males except for slight blanching. In combination with thiouracil, however, it appeared to confer on chemically hypothyroid birds some more normal aspects of plumage development. With increasing dosage, growth rates of anterior breast, posterior breast, back, and saddle approached levels characteristic of normal rather than hypothyroid animals. At 0.2 mg acetylthyroxin,

comparative rapidity of growth in different tracts resembled untreated more than thiouracil controls. At 0.1–0.15 mg, posterior breast and (to a slighter extent) saddle feathers showed increased barbulation and black melanin in previously red, lacy feathers, extending to anterior breast at 0.1 mg. Both birds reverted to red, hackled feathers after acetylthyroxin was discontinued. Tipped, barred, or complete blanching occurred at 0.2 mg.

186. DIFFERENTIAL MECHANISMS OF PITUITARY ACTIVATION BY CHEMICAL AGENTS.
Charles H. Sawyer and J. E. Markee, Duke University. (Lantern; 15 min.)

Copper salts and convulsant drugs such as picrotoxin induce ovulation in the rabbit by stimulating LH release from the adenohypophysis. It has been generally agreed that the chemicals exert their stimulating effect on the central nervous system and that activation of the hypophysis is indirect. The present study, which employs 150 mature female rabbits, introduces evidence that, whereas picrotoxin definitely functions by way of the nervous system, at least a large part of the copper stimulus is exerted directly on hypophyseal cells.

Picrotoxin-induced ovulation was always preceded by convulsions; in their absence pituitary activation failed to occur. Reduced dosages of picrotoxin led to extremely severe convulsions in rabbits previously treated with Dibenzamine or atropine, but ovulation followed in only one case in 10 animals so treated. These results are interpreted as evidence that the nervous stimulus was generally blocked from reaching the hypophysis. Nembutal in anaesthetic doses blocked the convulsive and pituitary-stimulating actions of doses of picrotoxin two to 4 times the minimal stimulating level.

Ovulation was induced by copper acetate in dosage which produced no overt signs of nervous stimulation. The copper stimulus to ovulation was blocked by neither the anti-adrenergic, the anti-cholinergic nor the anti-convulsive agent. Finally, pituitary activation was achieved by injecting tiny amounts of copper acetate directly into the adenohypophysis.

Preliminary treatment with estrogen facilitates both neurogenically and copper-induced ovulation. It is suggested that the steroid acts at the hypophyseal level by lowering thresholds to both neurohumoral and direct chemical stimulation.

Embryology (Section C)

187. HYDROGEN PEROXIDE AS A SOURCE OF OXYGEN FOR ASPHYXIATED NEWBORN GUINEA PIGS.¹ James A. Miller, Faith S. Miller and Carolyn B. Farrar, Emory University. (Lantern; 15 min.)

Tripp's successful use of H_2O_2 in treating anaerobic peritonitis in guinea pigs (1946) suggested the possibility of its use as a source of oxygen in asphyxia neonatorum.

In preliminary experiments day-old or younger animals injected intra-peritoneally with 3% H_2O_2 were able to survive exposures to 95% N_2 and 5% CO_2 which were lethal to water-injected littermates.

In Series II both experimental and control animals were exposed until dead, and the last gasp recorded as time of death (T.O.D.). Two and one-half cubic centimeters H_2O_2 /100 gm increased survival approximately 40% over controls. Five cc/100 gm caused respiratory embarrassment from excessive intra-peritoneal pressure. One of 10 such animals died before testing and another was saved by a stab wound which allowed escape of some gas. Discarding those in which pressure was extreme, the 5 cc animals average slightly longer than the $2\frac{1}{2}$ cc; including all animals tested they averaged slightly shorter.

Animals injected subcutaneously with H_2O_2 were more variable, but generally survived longer than did those in the intra-peritoneal series.

The effect of H_2O_2 upon recovery from sublethal exposures was also studied. Stages in recovery used for timing were the reappearance of breathing, attempt and completion of the righting reflex and crawling. Of these the most reliable was the first attempt to right. Hydrogen peroxide consistently decreased the time of appearance of each of these reflexes.

Confirmatory experiments performed on 300 gm animals gave similar but less consistent results.

It is concluded that 3% hydrogen peroxide, injected intra-peritoneally prolongs survival and speeds recovery from sublethal exposures to anoxia in the guinea pig.

¹Supported by a grant (No. RG281) from the Division of Research Grants and Fellowships of the National Institutes of Health.

188. DEVELOPMENT OF *DASYBRANCHUS CADUCUS* FROM EGG TO PRE-ADULT. C. G. Bookhout, Duke University and Lerner Marine Laboratory. (Lantern; 15 min.)

Eggs are deposited in plum-shaped jelly masses, become ciliated gastrulae in 8 hours, and trochophores in 24 hours. The latter is characterized by two red eyes, apical tuft, and green pigmented bands between a prototroch and telotroch. At two days a neurotroch appears, and at three days larvae emerge from the jelly to become part of the plankton. They are positively phototropic and negatively gestropic. After 7 days they settle; at 9 or 10 days they lose their cilia and undergo metamorphosis. Development of fertilized eggs removed from the coelomic cavity shows that jelly is not necessary for early development, nor does it hasten metamorphosis.

The best indication of age of larva and stage of juvenile is the condition of the setae. A notopodial and neuropodial winged seta develops simultaneously on each side in segments 2-4 at the 4th or 5th day. By the time of metamorphosis there are two winged setae per bundle in segments 2-4, and a single notopodial and neuropodial hooded uncinus on each side in segments 5-7. Post-larval development involves the occurrence of winged setae and the loss of uncini in thoracic segments. An increase in uncini in abdominal segments results in 4 tori per segment. The differentiation of sense organs, digestive system and other internal organs is discussed.

189. OBSERVATIONS ON CLEAVAGE AND EARLY GERM LAYERS IN *ANOLIS CAROLINENSIS*. George W. D. Hamlett, Louisiana State University School of Medicine. (Lantern; 15 min.)

Cleavage of the *Anolis* egg begins in a protoplasmic disc on the surface of the yolk. It is meroblastic at first; and produces a blastoderm resembling in surface view that of the birds. The central cells, however, are not cut off from the underlying yolk until relatively late, so that a distinct segmentation cavity is not formed, and nuclei from the periblast and the central cells pass freely into the yolk. By the 500 cell stage the blastoderm is composed of a single layer of surface cells, but with many "free" nuclei in the underlying yolk. These nuclei undergo mitosis, and there is an apparent attempt to organize the yolk into blastomeres. Some of those nuclei at the surface of the yolk, just beneath the blastoderm, appear to succeed in organizing large yolk-laden cells which are added to the blastoderm. This eventually becomes bilaminar, the compact surface layer being composed of smaller yolk-free cells, the lower layer made up of larger, irregularly arranged yolk-rich cells, many or most of which have been formed from the nucleated surface of the yolk or from the deeper zone of the marginal periblast. These layers are apparently extraembryonic ectoderm and entoderm; the primitive plate, from which develop the notochordal canal and primitive streak (and embryonic entoderm?) arises in close connection with the surface layer here identified as ectodermal. In the processes of notogenesis *Anolis* is closer to mammals than to birds.

190. AN ANALYSIS OF THE ROLE OF THE APICAL RIDGE OF ECTODERM IN THE DEVELOPMENT OF THE LIMB BUD IN THE CHICK.¹ John W. Saunders, Jr., The Johns Hopkins University and the University of Chicago. (Lantern; 15 min.)

At the apex of the early limb bud the ectodermal cells are oriented radially over the mesoblast, forming a thickened ridge (nipple-like in cross section). Excising this ridge in 72-96 hour embryos results in a limb deficient in terminal parts (Saunders, '48). Inserting thin strips of mica (ca. 30 μ) between the apical ectodermal ridge and subjacent mesoderm for periods longer than approximately one and one-half hours is followed by flattening of the ridge and development of defective limbs.

To test whether the ectodermal ridge has a role in establishing the specific type of limb (wing or leg) which is laid down during development of the bud, or if it affects its axes of symmetry, the ectodermal cap was excised from leg buds of 72-84 hour embryos, and the underlying mesoderm grafted immediately subjacent to the apical ectoderm of the wing bud. Of 16 cases, 11 showed chimaeric appendages consisting of proximal wing parts and distal leg parts, i.e., toes; 5 grafts formed only disorganized masses of tissue. In three of the former cases, the grafts were inserted with antero-posterior and dorso-ventral axes reversed. The leg parts formed from them were oriented according to the original axes of the graft.

These results indicate that the normal orientation of cells in the ectodermal ridge is essential for development of a typical limb. The ridge apparently exercises no influence, however, on the specificity or symmetry of the limb parts.

¹Work aided by the Abbott Memorial Fund of The University of Chicago, and by The American Cancer Society through the Committee on Growth.

191. DEVELOPMENT OF INTESTINAL COILING IN THE ANURAN TADPOLE. Norman E. Kemp, University of Michigan. (Lantern; 15 min.)

Experiments on embryos and larvae of *Rana pipiens* have elucidated some of the factors governing the morphogenesis of intestinal coiling. Factors considered in this study are: digestion of yolk, elongation of the digestive tract within the limited confines of the enlarging pleuroperitoneal cavity, maintenance of the tubular shape of the tract, anterior and posterior attachments and dorsal mesentery of the tract, nerve and blood supply. Three series of experiments were performed on tailbud stages: (1) transections of embryos at various antero-posterior levels; (2) ablation of ventral pieces of embryos, including all or parts of midgut and hindgut; (3) ablation of dorsal pieces, including all or portions of the neural tube and notochord. A 4th series consisted of opening the body cavity of larvae at stages after coiling had commenced or been completed.

Results of these experiments have shown that the utilization of stored yolk is greatly retarded, coiling is inhibited, and the digestive tube tends to flatten if circulation of blood to the intestine is not established. Coiling is inhibited, furthermore, in tracts which, as a result of transection, come to end blindly within the coelom. Regulation to normal form may follow extensive deletions of entoderm of the midgut or hindgut. Intestinal coiling may develop normally in embryos lacking the entire nervous system and notochord. Opening the body cavity results in unwinding of any coils already present.

192. OVULATION IN THE MOUTHBREEDING CICHLID FISH, *TILAPIA MACROCEPHALA* (BLEEKER). Lester R. Aronson and A. Marie Holz-Tucker, American Museum of Natural History. (Lantern; 15 min.)

Although ovulation has been known for many years as a definite process in teleost reproduction, relatively little attention has been given to this stage of the ovarian cycle. In contrast to those of other vertebrates the ovaries of most teleosts are covered by an ovarian wall which prevents the ovulated eggs from falling into the coelom. In some teleost species a definite lumen is present, and in the Cyprinodont *Oryzias latipes* the eggs have been described by Robinson and Rugh as dropping into this space. In the mature unovulated ovaries of *Tilapia* and many other teleosts no lumen can be recognized either macroscopically or when the ovaries are sectioned and examined microscopically. During ovulation in *Tilapia* the very elastic follicles rupture in an area of non-vascularized tissue to the side of the germinal disc, and the follicle walls shrink toward the mid-line of the ovary carrying with them the interstitial tissue and immature ova. Thus

a lumen, which is continuous with the oviduct, is formed on the lateral side of each ovary. The time of ovulation can be estimated with a fair degree of accuracy from the pattern of sexual behavior. Preliminary observations indicate that the individual ovum may ovulate within a minute or two. For the entire batch of mature eggs, ovulation may take 10 to 15 minutes. Egg laying generally occurs one to two hours after ovulation and sometimes much sooner. Slides showing the actual process of ovulation will be shown.

193. THE REPRODUCTIVE POTENTIAL OF A SINGLE CLONE OF PELMATHYDRA OLIGACTIS. C. L. Turner, Northwestern University. (Lantern; 15 min.)

A single animal provided with optimal food conditions produces as many as 35 consecutive buds and remains in an active reproductive condition at the end of a 30 day period. Food conditions can be controlled so that no buds are produced. Under most favorable conditions 6 buds of different ages are attached to the parent at one time and in one instance a new bud had formed upon a bud 40 minutes before its detachment.

Seven generations of new individuals can be produced within the 30 day period. There is no evidence of decline in rates of reproduction or vigor up to the 4th generation, but a physiological depression begins to appear during this generation. The depression, marked by inactivity, failure to feed and grow and apical disintegration is progressive in succeeding generations and relatively few lines of the strain survive beyond the 7th generation.

Under optimal food condition over 100,000 individuals will be produced by budding in less than 60 days.

194. A COMPARISON OF THE EFFECTS OF SODIUM AZIDE, THIOUREA, MALEIC ACID, AND MALONIC ACID ON DEVELOPMENTAL PATTERN IN THE SAND DOLLAR. Olin Rulon, Northwestern University. (Lantern; 15 min.)

Newly fertilized eggs and 6-hour blastulae of *Dendraster excentricus* were exposed to critical concentrations of the above agents continuously and for varying intervals of time (followed by return to sea water).

Azide, at certain critical concentrations, caused as high as 90% exogastrulation. Thiourea caused at most 20% exogastrulation. Maleic and malonic acids were not effective agents in the production of exogastrulae but both were effective (under certain conditions) in causing the development of highly active, ciliated regions and "knobs" at the apical end of the larva. Under certain conditions maleic acid caused the development of a large number of differentiated radial plutei. In these experiments neither azide nor thiourea were effective in causing the last mentioned modifications.

The differences in action of these agents may lie in the degree of differential inhibition, conditioning, or recovery in the gradient system of this egg where cytochrome oxidase and probably other oxidative enzymes are important gradient correlates.

195. ACID AND ALKALINE PHOSPHATASE ACTIVITY OF THE YOLK AND YOLK SAC OF THE DEVELOPING CHICK EMBRYO. Liselotte Mezger, Washington University. (Introduced by Florence Moog.) (10 min.)

Using a modified King-Armstrong method, the acid and alkaline phosphatase activities of the yolk and yolk sac (plus some adhering yolk) of chick embryos from one to 19 days of incubation were measured in milligram phenol liberated per milliliter per 30 minutes. The alkaline phosphatase activity found in both the yolk and yolk sac was low, at no time exceeding one-tenth the maximum in the developing chick from two to 12 days or one-six-hundredth that of the intestine in three-week-old chicks (Moog, '46). The acid phosphatase of the yolk showed a comparably low activity. However, in the yolk sac acid phosphatase not only showed a maximum activity equal to the highest found in the functioning organs of three-week-old chicks but about 5 times that to be found in the embryo itself from two to 12 days. The activity per milliliter rises rapidly from the first to 9th days, declines slightly and rises again on the 17th day. Calculated per unit of dry weight, the rise in activity from one to 9 days is even more striking, although the general picture remains the same.

Since yolk is utilized by the embryo for its development and since there is evidence that phosphatases may be active in transporting systems, it is postulated that this is the role of acid phosphatase in the chick yolk sac; it may also function as a digestive enzyme.

196. THE CRITICAL INHIBITORY VOLTAGE THEORY OF GROWTH LIMITATION: FURTHER EVIDENCE. Gairdner Moment. Goucher College. (Lantern; 12 min.)

The anatomical basis of this theory has recently been challenged on the ground (1) that Sun and Pratt ('31) were wrong in claiming that earthworms possess the full adult number of segments at hatching, and (2) that earthworms do not have a limited number of segments as was asserted (Moment, '46) because increase of segments continues throughout life.

The number of segments in 300 newly hatched *Eisenia foetida* identified and counted under a dissecting binocular microscope fully confirms Sun and Pratt.

Serial sections of 90 worms cut at intersegment 50/51 and fixed at 4 intervals from 8 to 20 days regeneration at 25°C. showed a regular increase in segments up to 87.2. Counts on 250 worms in 4 groups from 20 to 60 days regeneration gave 90.39, 90.4, 91.3, and 89.5 segments respectively. Thus the formation of new segments in regeneration from 50/51 stops at about 20 days at 25°C. Regeneration of voltage follows segment number, not growth in millimeter which is a much slower process continuing far longer.

197. THE USE OF PLACENTAL AND OVARIAN SCARS IN DETERMINING REPRODUCTIVE HISTORY. H. T. Gier, Kansas State College. (10 min.)

Examination of large series of fresh reproductive tracts of foxes and coyotes has shown a very high correlation between: (1) corpora lutea and attached embryos; (2) corpora rubricans and placental scars; and (3) pre-season enlarged

follicles, maturing follicles, and corpora lutea. Placental scars are distinguishable for at least two years following parturition but fade rapidly in formalin preservatives. Corpora rubricans are not consistently distinguishable for more than one year and are not faded by fixation.

198. EXPERIMENTAL STUDY OF ARTIFICIAL PARTHENOGENESIS IN THE FROG. John R. Shaver, University of Missouri. (Introduced by M. J. Guthrie.) (15 min.)

Experiments have been continued on the "second factor" activity of cytoplasmic granules from adult and embryonic tissues in artificial parthenogenetic activation of frog eggs. The granules sedimentable from frog blood cell extracts or from frog serum at from 3,000 to 60,000 $\times g$ account for a large part of the "second factor" activity of the cells from which they were extracted, or of the whole serum, in *Rana pipiens* and *Rana fusca*. Granules sedimentable at lower centrifugal forces are more effective second factor substances than smaller particles. Similar results have been obtained in *Rana fusca* by centrifugal fractionation of extracts of testis, brain, lung, muscle and liver. Heparin appears to inhibit parthenogenetic cleavage, although it does not affect the second factor activity of granules sedimentable from extracts of heparinized blood. In *Rana pipiens* second factor activity does not appear in embryonic extract granules until 18-19 hours at 20°C. Heat (60°C.), ribonuclease destroy the second factor activity of granular suspensions. Blockage of embryonic development due to thermal shock, oxygen pressure, or parthenogenetic activation does not affect the activity of granules from the blocked embryos; reversible blockage by sodium azide produces an apparently reversible blockage in the second factor activity of granules from treated embryos. There appears to be a general correlation between the second factor and thromboplastic activities of granules from extracts of adult tissues. Granules from yeast extracts, and purified nucleic acids, are inactive.

199. ANTIGENIC ACTIVITY OF CYTOPLASMIC GRANULES FROM EMBRYONIC TISSUE. John R. Shaver, University of Missouri. (Introduced by Daniel Mazia.) (10 min.)

The purpose of these experiments, which were done in collaboration with Dr. P. Bordet, Pasteur Institute, Brussels, Belgium, was to test the relative antigenic activities of cytoplasmic granules of different sizes isolated from homogenates of frog embryo cells by differential centrifugation; 2.5 gm of just-hatched larvae of *Rana fusca* were crushed in M/200 phosphate buffer, pH 7.4; the brei was cleared of cellular debris, nuclei and pigment; and the resulting supernate (A) was centrifuged at 17,500 $\times g$ for 20 minutes (Fraction I); at 60,000 $\times g$ for 10 minutes (Fraction II); and at 60,000 $\times g$ for 60 minutes (Fraction III). The granules were washed, suspended in a definite volume of phosphate buffer and, together with the final supernate (Fraction IV), injected into rabbits, the sera of which had tested negative for reactions to the fractions prior to injection. Aliquots were removed from the suspensions for total nitrogen determinations. Expressed as percentages of A, these values were: I-18, II-8, III-9 and IV-64. After 12 injections the animals were killed, bled and the sera

prepared and tested against identically prepared antigenic fractions. Positive precipitation and ring tests were obtained with all antigen antisera combinations. However, Fraction I antiserum gave reactions 2-3 $\times$ stronger with all the antigenic fractions than did the other antisera. The significance of these results for the possible role of cytoplasmic granules in protein synthesis is indicated.

Cellular and General Physiology (Section D)

200. THE EFFECT OF ENVIRONMENTAL TEMPERATURES ON CELLS. I. EFFECT UPON THE MORPHOLOGY AND VIABILITY OF NEOPLASTIC CELLS. Lawrence B. Walsh, Herman T. Blumenthal and Donald Greiff, St. Louis University. (Introduced by Walter C. Randall.) (15 min.)

Minced tumor tissues — Sarcoma 37 — was stored at 0°C.; — 30°C.; — 70°C.; and — 183°C. for various periods of time and then injected into mice. The effect of these temperatures on the morphology and transplantability of tumor cells was determined. Studies of the effect of numerous freezings and thawings were also made. The histological picture of this tumor is that of a polymorphous, very invasive sarcoma, containing little or no connective tissue stroma. Fragments of tumor cells subjected to the low temperatures enumerated for varying periods and fixed and starved immediately after thawing showed no definite morphological change.

Two hours after transplantation the cellular picture shows normal appearing tumor cells as well as the beginnings of cellular deterioration. This cellular disintegration continues up to 120 hours after inoculation at which time it was not possible to find histologically normal appearing tumor cells. At 7 days peripheral regions of the transplant showed rapidly dividing tumor cells. By 12 days the picture was one of a typical Sarcoma 37. This was noted in transplants of tumor subjected to low temperature as well as those immediately transferred from donor to host.

In the strains of mice used in these experiments a high degree of specificity was noted. However, a first transplantation to any of the strains of animals employed always resulted in good tumor growth. After subjection to low temperatures it was impossible to transplant the tumor successfully for more than a few transplant generation in some of the strains.

201. THE EFFECT OF PRIOR INJECTIONS OF LYOPHILIZED NORMAL MOUSE TISSUES ON THE SUBSEQUENT GROWTH OF A TRANSPLANTED TUMOR.¹ Nathan Kaliss² and G. D. Snell, Roscoe B. Jackson Memorial Laboratory. (Lantern; 15 min.)

Snell et al. (1946, 1948), using inbred mice, have shown that prior injections of a lyophilized tumor will enhance the growth of homoio-transplants of the tumor where it would normally regress. In the present investigation, injections of lyophilized normal liver, kidney, spleen, washed red blood cells, and adenocarcinoma 15091a, from inbred strain A mice, were given to strains C57 black and C57 brown mice prior to subcutaneous implantation of live strain A tumor 15091a. In the controls (no prior injections) the tumor regressed in all of 33 black and 10 brown mice. Of the C57 black and brown mice injected with

lyophilized tissue, as indicated, the following numbers died with positive growths of tumor 15091a: Strain A *liver*, 8 out of 31 black, 2 out of 10 brown; *kidney*, 13/30 black, 6/10 brown; *spleen*, 9/14 black, 9/10 brown; *red cells*, 0/24 black; tumor 15091a, 28/35 black, 5/9 brown; strain C *kidney*, 1/18 black; strain P *kidney*, 0/22 black. It is thus demonstrated that the growth enhancing agent (or agents) for the homoiotransplants is present in normal tissues, as well as in the tumor.

¹Supported in part by a grant-in-aid from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council, and in part by a grant to the Roscoe B. Jackson Memorial Laboratory from the National Cancer Institute.

²Senior Fellow in Cancer Research of the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

202. SYNERGISTIC AND ANTAGONISTIC INFLUENCES ON COLCHICINE-POISONED SEA-URCHIN EGGS. Ivor Cornman, George Washington University. (Introduced by A. F. Shull.) (7 min.)

The antagonism between inositol and colchicine first demonstrated in plant cells (Chargaff *et al.*, *Science* 108: 556) applies also to dividing eggs of the sea urchin, *Lytechinus* (Cornman, in press).

Glucose at 8–32 mM/L will lessen the effects of 0.01–0.02 mMolar colchicine when added simultaneously 10 minutes after fertilization. The large amount of glucose suggests that the antagonism results from increased energy supply rather than from competition for enzymes. This energy could: (a) supplant energy no longer available from the colchicine-poisoned system; (b) inactivate the colchicine. Several kinds of evidence give the second possibility a slight advantage. NaF at 6–8 mMolar slightly increases the colchicine effect, and is not affected by glucose until enough is added to abolish the colchicine effect as well. NaF alone at 10 times this concentration barely slows cleavage.

Animal cortical hormone antagonizes colchicine (Lettré, *Naturwiss* 33: 283; Törö, *Arch. exp. Zellf.* 22: 1). Chloroform (Levan and Steinegger, *Hereditas* 33: 552) and phenylurethan (Deysson, *C. R. Acad. Sci.*, 220: 367) are synergistic with colchicine. Mitoses in excised *Colchicum* roots are blocked at metaphase by 10% colchicine (Cornman, *Bot. Gaz.* 104: 50), but attached roots are unaffected by 20% colchicine (Levan, *ibid.*). These observations are compatible with the idea that all cells can to some extent inactivate colchicine through a system which is inhibited by NaF, CHCl_3 , or phenylurethan, or by mechanical interference with the source of nutriment, and is aided by glucose or cortical hormone.

203. THE PRODUCTION IN MICE OF ANTIBODIES AGAINST MOUSE MITOCHONDRIA AND MICROSOMES.¹ G. D. Snell, Roscoe B. Jackson Memorial Laboratory. (10 min.)

Transplantable mammary gland spindle-cell carcinoma 15091a, A strain origin, grows temporarily in C57 black mice and then regresses. C57 black mice were given three or more successive inoculations of the tumor and bled about 10

days after the last inoculation. When tested by the collodion particle technique, the antisera thus prepared reacted with A strain liver washed microsomes and, less strongly, with 15091a microsomes. There was little if any reaction with mitochondria. However, an antiserum which agglutinated Ak strain liver mitochondria was prepared by injecting these mitochondria, plus killed tubercle bacilli as an adjuvant, into B alb C mice. This same technique has not given a good antiserum against microsomes.

¹ This work was supported in part under a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and in part under a grant to the Rosecoe B. Jackson Memorial Laboratory from the National Cancer Institute.

204. INTERACTIONS BETWEEN NUCLEUS AND CYTOPLASM. William R. Duryee, National Cancer Institute, National Institutes of Health. (Lantern; 10 min.)

Experiments on ovarian amphibian eggs have shown that typical nuclear injury, usually observed in 2-5 days following 3000 r doses of X-rays, may be delayed up to two weeks by low temperatures (5°C.). Longer exposures to cold have allowed cytoplasmic damage to appear before nuclear degeneration set in. However, on return of animals to room temperature pyknosis ensued. Microinjections of irradiated cytoplasmic material into non-irradiated cells result in typical pyknosis in 15-30 minutes. Conversely, sections of eggs irradiated with 3000 r and similarly treated, all showed extensive infiltrations of nuclear substance into the cytoplasm. It must be concluded that normally cytoplasmic factors stabilize the nuclear colloids and that abnormally large amounts of nuclear material can be extended through the nuclear membrane.

205. ADAPTATION OF *ESCHERICHIA COLI* TO USE OF FOUR-CARBON DICARBOXYLIC ACIDS. Thomas C. Nelson, Department of Zoology, Columbia University. (Introduced by F. J. Ryan.) (15 min.)

In an attempt to screen for biochemical mutants affecting the Szent-Gyorgyi cycle of *E. coli* K 12 it was found that variable growth occurred on the 4-carbon dicarboxylic acids. The original strain would not grow in synthetic medium containing the acids if the inoculum consisted of less than about 10^4 bacteria. If larger inocula were used adaptive growth occurred with a long and variable lag period. Strains established by isolation from adapted cultures grew with a short and constant lag period when single bacteria were inoculated. Adaptation to one acid resulted in simultaneous adaptation to the others. The adapted strain was termed s^+ and the original strain s^- in accordance with a similar phenomenon in *Moraxella*. The s^+ strain maintained its ability to grow in acids after repeated culture in glucose. Contamination by an acid-utilizing bacterium as an explanation was eliminated by appropriate tests.

Attempts to demonstrate mutation of s^- to s^+ by the method of plating and variance analysis have failed. No s^+ cells have been detected in s^- cultures although samples of the s^- cultures produce growth in liquid medium. Slow growth in liquid and subsequent mutation similar to the situation in *Pseudomonas* has not been eliminated as an explanation.

Manometric studies have shown the QO_2 of s^+ cells to be three to 5 times greater than s^- cells in succinate and 8 to 10 times greater in fumarate and malate. Further studies on the biochemical identification of the adaptive block are in progress.

206. IN VITRO TRANSFER OF RADIOIRON ACROSS THE NUCLEATED AND NON-NUCLEATED ERYTHROCYTE MEMBRANE.¹ L. M. Sharpe, P. S. Krishnan and J. R. Klein. (Lantern; 12 min.)

Whole blood incubated *in vitro* with radioiron, as ferric ammonium citrate, overnight at 37°C. showed increased accumulation of iron in the washed erythrocytes in the following ascending order: man, dog, rat pigeon, duck. Addition of NaCN, final concentration of 0.005 M, inhibited the transfer almost completely.

The uptake of iron by duck erythrocytes was studied in some detail. Incubation of whole blood with radioiron resulted in a progressive increase in the radioactivity of the red blood corpuscles which reached a peak at 6 hours and then tended to decline somewhat when incubation was extended to 24 hours. Erythrocytes, which had taken up the optimum amount of iron following incubation, were separated from plasma, washed with saline, hemolysed, the debris removed and the supernatant treated with trichloroacetic acid. Radioactivity measurements of cell debris, protein, and nonprotein fractions showed that about three-fourths of the total activity was associated with the protein fraction.

Uptake of iron and incorporation into protein occurred also if the radioiron was added after hemolysis of the cells, but was negligible if added to the centrifuged solution of the cell contents alone.

¹Research carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

207. GIANT MITOCHONDRIA IN THE FLIGHT MUSCLES OF INSECTS. Mary Ishimoto and Carroll M. Williams,¹ Harvard University. (Introduced by Alfred S. Romer.) (Lantern; 15 min.)

The flight muscles of the higher insects are exceptional in that the spaces between adjacent fibrils within the muscle fibers are packed with rows of relatively large, ovoid bodies, which by analogy with the minute granules of ordinary muscle, have been termed "sarcosomes." Information concerning these prominent bodies has, hitherto, been limited to a knowledge of their existence. On this account, we have made a detailed study of the sarcosomes in the flight muscles of *Drosophila funebris* and the blowfly, *Phormia regina*.

(1) Flight muscle, characterized by giant sarcosomes, occurs only in the great indirect wing muscles of adult Diptera and Hymenoptera; (2) when the muscle is teased, the sarcosomes are liberated as a turbid suspension; (3) such isolated sarcosomes show pleomorphism, but are generally round to oval with an average diameter of 3.5μ ; (4) they may be isolated in any desired quantity by grinding the thoraces of flies in 0.2 M sucrose, filtering through muslin, and centrifuging at 2200 RPM for 5 minutes; (5) spectroscopically, the isolated sarcosomes show high contents of cytochromes *c*, *b*, and *a*; (6) manometric studies reveal significant titers of cytochrome oxidase, catalase, and succinic, malic, and alpha-

glycerophosphate dehydrogenases; (7) xanthine, glycine, alcohol, and choline dehydrogenases and choline esterase are apparently absent.

In view of their intracellular position, osmotic behavior, chemical composition, and high titers of respiratory enzymes, we are persuaded to regard the sarcosomes as the mitochondria of this highly specialized tissue.

¹This study was aided by the Lalor Foundation and the American Cancer Society.

208. GLYCOGEN CONTENT OF VARIOUS ARCTIC ANIMALS. B. J. Sullivan, Boston College, and X. J. Musacchia, St. Louis University. (Introduced by Sr. Elizabeth Seton MacDonald.) (15 min.)

Estimations of the quantity of glycogen in the tissues of various Arctic animals were carried out at the Arctic Research Laboratory in Point Barrow, Alaska, under the auspices of the Arctic Institute of North America and the Office of Naval Research. The tissues of birds, mammals, fish, and invertebrates were analyzed for glycogen using a modified Plueger method. The method involved digestion of the tissues with alkali, deproteinization, acid-hydrolysis, and estimation of the amount of glucose in the hydrolysate by means of the Somogyi-Shaffer-Hartmann method.

The following animals were investigated: Sabine gull, Loon, Jaeger, King Eider, Arctic owl, tal, ground squirrel, blackfish, whitefish, sculpin, Arctic cod, sea cucumber, sea urchin, and crab. Liver, kidney, and muscle tissue were analyzed; in work on the invertebrates, the entire organism was digested and analyzed. Whenever possible, a comparison was made between juvenile and adult forms.

At present two phases of research are being followed: further analyses of preserved tissues, and a recheck of the methods used. Further development of this line of research will consist in an extension of the investigation on Arctic animals, and a comparison between Arctic animals and closely related forms in the temperature zone.

209. SURFACE AREAS OF CHROMOSOMES IN RELATION TO THEIR ACTIVITY. Edward O. Dodson, University of Notre Dame. (Introduced by J. D. Mizelle.) (10 min.)

Calculations of specific surface (square meters per gram) were made for mitotic chromosomes from ovarian connective tissue of *Amphiuma* and for the lateral loop chromosomes of the primary oocytes of the same animal. The figures are 2.12 and 108.37 m² per gram respectively. There was no reason to expect unusual activity in the connective tissue, while the oocytes were intensively synthesizing nucleolar material, yolk, and formative substances. Thus the higher specific surface of the chromosomes goes with the more active cells. Activity of organic catalysts is proportional to their specific surfaces, and the specific surfaces of some synthetic catalysts are of the same order as that of the lateral loop chromosomes. This is consistent with the accepted theory that the chromosomes function as catalytic agents, presumably in the formation of enzymes.

210. EFFECT OF TEMPERATURE ON THE LIPIDS IN THE BLOOD OF *CHRYSEMYS PICTA*. Joel R. Lieb and Charles G. Wilber, St. Louis University. (Introduced by B. Luyet.) (15 min.)

The effect of ambient temperature on the fatty acids, cholesterol, and lipid phosphorus in the blood of the turtle was studied. The specimens were exposed to a given temperature for 24 hours. Turtles exposed to 32°C. showed an increase in blood fatty acids (as compared with controls kept at 22°C.) followed by a decrease after exposures to 38°C. Exposure to 32°C. resulted in no change in blood cholesterol but a significant decrease obtained after exposures to 38°C. There is a slight decrease in lipid phosphorus after exposure to 32°C. and a marked drop after exposure to 38°C. If the suggestion in the literature that the ratio, cholesterol/lipid phosphorus, is an index of water content of the tissue in question is valid, the present results suggest that the water content of the blood of turtles is greater at 32°C. than at 22°C. or 38°C. Further, if it is generally true that among vertebrates cholesterol content of the blood varies inversely with the basal metabolic rate, the present work indicates that the BMR of the turtles increased after exposure to 38°C. but was unchanged after exposure to 32°C.

211. EFFECT OF CARBON DIOXIDE ON INSECT ELECTRORETINOGRAMS. Theodore L. Jahn and Lewis A. Kaplan, University of California at Los Angeles. (Lantern; 7 min.)

The electrical response of the dark adapted eye of various insects to repetitive stimulation by intense half-second light flashes was recorded. Variation of this response with adaptation was noted. The insects were then placed in various percentages of CO₂, O₂ and N₂, and the retinograms similarly recorded.

In air, body movements were frequently imposed upon the electroretinogram. These disappeared rapidly in 80% CO₂ and 20% O₂.

For the sphinx moth, *Celerio lineata*, which has a rather complicated retinogram, adaptation in air decreases the response generally, and the c-wave the most. In 80/20/0 (CO₂, O₂, N₂) the d-wave and after potential disappear first leaving a negative square wave which decreases to zero as the CO₂ percentage is raised to 100%. These effects are wholly reversible. Pure N₂ reduces the retinogram to zero but without the other effects of CO₂.

Light adaptation of *Papilio daunus* in air causes a heightening of the c-wave only. In 80/20/0, b- and d-waves disappear and the remaining square wave increases to about double the average value in air. This then decreases and fluctuates near a value of 120% of normal. Ether has a similar but slower effect. On insects with simpler retinograms, the general effect of CO₂ is to diminish the response.

The effect of CO₂ can be explained by assuming differential effects on the responses of the eye and optic ganglion which are apparently summed algebraically is the electroretinogram.

212. THE BLASTOCOEL FLUID IN AMPHIBIAN GASTRULATION. Louis T. Stableford, Lafayette College. (Introduced by B. W. Kunkel.) (Lantern; 10 min.)

Various authors have considered the blastocoel fluid to play an active part in amphibian gastrulation. On the basis of Buytendijk and Woerdeman's ('27) pH values for blastocoel fluid in *Triton taeniatus* and those of Richards ('40) for capsular fluid in *Amblystoma punctatum*, Holtfreter ('44) suggested a surface tension gradient between blastocoel fluid, cells, and capsular fluid as the important factor. He used the behaviour of isolated cells and tissue fragments shock-treated with strong alkali to show morphogenetic significance for a high pH in the blastocoel fluid.

To ascertain directly any gradients, blastocoel and capsular fluids were collected from *A. punctatum* and *A. jeffersonianum* blastulae and gastrulae. The pH of the fluids was measured electrometrically and photoelectrometrically; surface tension was measured with the duNouy tensiometer. Existence of a pH gradient with high alkalinity in the blastocoel was confirmed; a correlated surface tension gradient was not.

Embryos, embryos with exposed blastocoel, and isolated vegetal hemispheres were cultivated in media of differing pH and surface tension. Neither a pH comparable to that of blastocoel fluid and nullifying the gradient, nor a low surface tension affected development of the preparations in any way. Aggregation of cells, wound healing, morphogenetic movements all occurred.

It was concluded that the blastocoel fluid in amphibians, as in echinoderms, serves a purely mechanical function and has no morphogenetic significance.

PAPERS PRESENTED BY TITLE

Animal Behavior

213. AN EXPERIMENTAL STUDY OF THE POSSIBLE SENSORY CUES INVOLVED IN THE RESPONSES OF ALLIGATOR MISSISSIPPIENSIS TO THE SOUNDS OF THE FRENCH HORN. J. A. Murnin and J. V. Quaranta, New York Zoological Society. (Introduced by T. C. Schneirla.)

The fact that *Alligator mississippiensis* responds not only to the sounds of the French Horn, but also to a wide variety of extra-musical stimuli was of especial interest to the present investigators. Apparently, any frequency, whether auditory or vibratory, which is of a small number of cycles, rhythmical, and of a jarring quality will elicit the roaring response.

A preliminary electrical analysis carried out with a ballistics galvanometer and utilizing audio-filters suggests that there are marked differences in energy output (measured in d.b. over 5 frequency ranges) between the sounds of the French Horn, for notes both two and three octaves below Middle "C," and the vocal responses of *A. mississippiensis*. There is a striking similarity in energy output between notes of the third Octave Below Middle "C" on the French Horn and notes of the second Octave Below Middle "C." Analysis of the vocal responses of *A. mississippiensis* yields the greatest number of d.b. for the frequency range: 100-250 cycles. For French Horn sounds, both two octaves and three octaves below Middle "C," the greatest number of d.b. is obtained for

the frequency range: 250-500 cycles. It would appear that identical or approximately equal energy outputs for the same frequency-ranges are not important in eliciting the vocal responses of *A. mississippiensis*. Another factor such as "wave-form" may provide the essential auditory clue.

Also preliminary sectioning of the ear suggests that extirpation techniques are entirely feasible in controlling strictly auditory cues.

214. NOTES ON THE SOCIAL BEHAVIOR IN A HERD OF NYALAS, *TRAGELAPHUS ANGASI* (GRAY.) Dieter Bueckhardt, New York Zoological Society. (Introduced by L. T. Evans.)

The observations were made on 11 Nyalas which were living under semi-natural condition in the Bronx Zoological Park. From mid-June to mid-September 1949 the herd was observed once a week for a period of 13 to 15 hours, and almost every day for short intervals. The animals could be recognized by individual color patterns.

The observations show that several factors are responsible for cohesion among the animals:

1. The dominant male may drive the animals together.
2. The herd may follow the oldest female (also the most dominant) with the dominant male following the herd.
3. An individual carries on physiological activities such as feeding, resting, and ruminating at the same time and place as others.
4. One animal may transmit his own emotion to the other animals by changing his appearance (raising the head to look for danger).
5. The animals tend to be gregarious.

Within the herd we find a dominance order at the head of which is a male. This order is produced by threatening or butting in frontal contact between two animals. Two animals show "positive contact" when they lick each other on the head. No correlation is evident between dominance order and corporal contact either in the feeding and resting situation, or in the licking behavior.

A female in heat rose from 5th to first place in the females' dominance order, but the oldest female continued to lead the herd. After estrus the original dominance order was resumed. The typical attitudes are depicted in sketches and photographs. They will be published together with the complete data in a future paper.

215. THE REPRODUCTIVE BEHAVIOR OF THE GIANT TORTOISES, *T. VICINA* AND *T. VANDENBURGHII*. L. T. Evans, New York Zoological Society.

The giant Galapagos tortoises in this country are becoming extinct because too few young are hatched to replace the oldsters. Only one egg was laid this year at the Zoo. It was infertile. However, 5 males were especially active in the herd at the Bronx Zoo. They were all observed to mount females although one *T. vicina* in particular was seen to copulate almost every day since last May.

No preliminary courtship was observed in any single case. The male approached a female from the rear. The concavity of his plastron fits snugly over the convexity of the carapace of the female, his forelegs on the forward surface of her

carapace; his head hangs forward and downward. Two minutes elapse while the penis comes into view momentarily and is inserted into the cloaca of the female. Suddenly he thrusts forward and upward and at that precise moment he roars while his rear feet are lifted off the ground. The roar is very low in pitch, lasts about one second, but may be repeated every 5 seconds (average) for as long as 6 minutes, usually, but two mountings last 26 minutes.

T. vandenburghii use the same posture but are more nervous, the head swings and the call is high and gasping and is repeated less frequently with the mouth wide open. In *vicina* males the mouth is almost closed while roaring.

216. THE VISUAL LEARNING OF *TESTUDO VICINA*. John V. Quaranta and L. T. Evans, New York Zoological Society. (Introduced by C. C. Little.)

Two giant tortoises, both male, were studied for their ability to learn in a series of visual discrimination tests.

Stage I: The experimental animal ($R =$) was conditioned to discriminate visually among three different pairings of orange, green and blue color papers of the same intensity. It was possible to obtain 100 trials on each pairing. All results were significantly above chance. During the course of the animal's conditioning process, it was possible also to transfer his positive inclination for orange to a positive inclination for blue. Thirty trials required. Learning was hastened by punishing for incorrect responses and rewarding for correct.

Stage II: Conducted to determine whether *T. vicina* was capable of blue-green discrimination, and hence that *T. vicina* actually saw the blue end of the spectrum. The validity of all previous discrimination-learning would thus be enhanced: the animal was not simply responding to a positive valence (reward orange) in stage I, but was actually choosing between a positive valence and a negative valence (no reward green or blue). Three greens of different intensities and different saturations were paired in every possible way with three blues of different intensities and different saturations. It was possible to obtain 162 successes out of 182 trials. The results are significantly above chance.

Stage III: Stage I was repeated for (reward orange), the control animal, with 32 trials. Results significantly above chance. Twenty-four trials required to change the positive valence of orange. Repeat stage II: in 81 trials, the animal's performance was significantly above chance.

General Conclusion: The principle of instrumental act conditioning (reward training) holds for *T. vicina*.

217. DIFFERENCES IN THE PATTERN OF SEXUAL BEHAVIOR IN LOW-DRIVE AND HIGH-DRIVE MALE GUINEA PIGS. William C. Young, University of Kansas.

Data were obtained from 161 10-minute tests of low-drive males that did not reach the point of ejaculation during the tests and from 82 high-drive males that ejaculated during the second half of the test. The behavior patterns during the first 5 minutes of the tests were compared. The patterns were regarded as

being composed of 5 measures that can be arranged in an ascending order with respect to strength of drive: (1) other behavior, a catch-all for indifference and any behavior that is not strongly sexual, (2) sniffing and nibbling, (3) nuzzling, (4) mounting, (5) intromission without ejaculation.

Except for one of 15 points of comparison, the percentage of the measures of low degree (other behavior, sniffing and nibbling, nuzzling) was higher during each of the 5 minutes in the low-drive than in the high-drive males. Without exception the percent of measures of high degree (mounting and intromission) was higher in the high-drive than in the low-drive males. During the tests of high-drive males mounting was the measure of behavior that was seen more often than any other during the initial excitement of the first minute, whereas nuzzling, regarded as a measure of lower degree, was seen more often during the tests of low-drive males. The difference is reminiscent of that encountered by Kinsey in men of high and low educational level, but data collected from the guinea pig indicate that in this species strain difference is involved.

218. STRENGTH OF SEX DRIVE IN THYROXINE-INJECTED MALE GUINEA PIGS. William C. Young, University of Kansas.

Six male guinea pigs whose strength of sex drive had been tested during 10 10-minute tests were injected with 0.1 mg thyroxine at 4- to 5-day intervals for 30 days. At the end of this time the weight loss was 10 to 15%. Tests similar to those made during the pre-treatment period were then resumed until 10 had been given. During the experimental period, injection of 0.1 mg thyroxine was continued at 4- to 5-day intervals which was sufficient to maintain the weight at the lower level. The control group was composed of 5 males given 10 tests each during the pre-treatment period and 10 tests during the experimental period.

Strength of drive of the most vigorous male was not affected by the treatment, neither was that of two of the least vigorous males. Decrease in strength of drive occurred in the other three experimental males, but it was no greater than the decrease in 4 controls. It is concluded therefore that an amount of thyroxine sufficient to lower the body weight of an adult male guinea pig 10 to 15% is without any readily detectable effect on the strength of its sex drive.

219. VOCALITY, A FACTOR IN THE ECOLOGY OF THE ALLIGATOR. L. T. Evans and J. Quaranta, New York Zoological Society.

A few writers have actually seen the *Alligator mississippiensis* (Daudin) roar in its native haunts. Their descriptions are similar in most details to those who have seen it bellow in captivity. All agree that it is a dramatic spectacle.

With the facilities of the Zoo at our disposal, we tried to induce the herd of 12 American alligators, the *Alligator sinensis* and the Nile Crocodile (all 14—large, fully matured specimens) to roar in response to their own calls played to them by tape-recorder and loud speaker, and to the French Horn direct and from recordings.

The recorded calls induced roaring and was the most reliable. The French Horn was almost as effective when played at E flat Concert (B flat), two octaves below middle C (confirming Beach, '44) and with a rhythm of a three-second blast and a two-second rest. However, roaring was elicited from one or more animals by other notes in the same octave. Only the alligators roared.

Abortive fighting, herding toward the sound (Horn, recording or alligator) and initial courtship behavior (the mutual rubbing of throat on back and neck while everting the gular scent glands), was induced by both the Horn and the recorder.

The extreme posture assumed by a roaring alligator, the deep resonant sound produced, the special ecological niche inhabited by the species, prompts us to compare it with other species which inhabit equally specific environments where territorial and mating calls must be sent great distances to be socially effective.

Cellular Physiology

220. GLYCEROPHOSPHATASES IN THE FROG KIDNEY. Thomas J. King and Ross F. Nigrelli. Department of Biology, Washington Square College, New York University and the New York Zoological Society.

It has been demonstrated for the first time, histochemically, that the normal kidney of the frog, *Rana pipiens*, possesses a strong alkaline glycerophosphatase reaction in its proximal tubules, especially at their brush borders, whereas the distal convolutions are negative. The glomeruli show a moderate alkaline reaction, categorizing the frog in a class with the cat (the only other animal thus far investigated that gives this reaction). All portions of the kidney gave negative acid phosphatase reactions, except for an occasional positive glomerular reaction due to the presence of this enzyme in the red blood corpuscles. Emphasis is placed on the fact that the results are based solely on the use of sodium-beta-glycerophosphate as the phosphoric ester hydrolyzed by the kidney phosphatase. It is quite possible that other substrates at different pH optima may show an entirely different phosphatase pattern.

221. THE EFFECT OF DDT ON THE ACTIVITY OF PHOSPHATASES IN VARIOUS TISSUES OF THE CHICKEN. Harold Burlington, Syracuse University. (Introduced by V. F. Lindeman.)

Beginning on the 8th day post-hatch, white leghorn cockerels were given daily subcutaneous injections of DDT dissolved in chicken fat. The dosage was increased gradually from 15 mg/kg body weight to 225 mg/kg. At approximately 88 to 97 days of age the animals were sacrificed and enzyme studies of different tissues were made. Total DDT administered was on the average 2.549 gm. A control group was maintained throughout the course of the experiment and tissues of these birds were analyzed for comparison with the treated.

The enzymes studied were acid (pH 5.0), and alkaline (pH 9.1) phosphatase, and adenosinetriphosphatase.

Liver tissue revealed no significant differences in these enzymes between the treated and control animals. Likewise, the acid and alkaline phosphatases of testicular tissue showed no difference in activity as a result of poisoning. How-

ever, there was a definite and constant variation between the ATP'ase activity in testes of treated and non-treated birds. The average activity of ATP'ase (measured by determining the number of μg of inorganic P liberated per milligram tissue per 15 min.) in the treated group was 3.707 μg P/mg/15 min., whereas the control group averaged 7.439.

This effect upon the activity of ATP'ase appears to result from a direct action of DDT.

222. THE RATE OF GLYCOLYSIS AND ATP'ASE ACTIVITY IN THE DEVELOPING CHICK RETINA. V. F. Lindeman, Syracuse University.

Between the 12th day of incubation and hatching time is the period during which the neural elements of the chick retina undergo maturation and differentiation. There exists a remarkably close parallelism between the rate of glycolysis and ATP'ase activity of the retina during this phase of development.

Composite curves of three series of experiments reveal the following values for ATP'ase activity (μg P released per 100 micrograms homogenized tissue per minute). Retina from embryos 12 days, 11.6; 14 days, 11.9; 16 days, 13.2; 18 days, 16.6; 20 days, 21.7; 22 days, 24. For lactate formation (μg lactate produced per 100 mg tissue per minute) the following values were obtained. Retina from embryos 12 days, 8.3; 14 days, 9.2; 16 days, 10.8; 18 days, 12.2; 20 days, 16.3; 22 days, 16.8.

It is to be noted that both the rate of glycolysis and ATP'ase activity increases about 100% during this phase of development in the retina. Experiments on more mature retina reveal no further change in either the rate of glycolysis or ATP'ase activity. It would appear that ATP'ase activity may be one of the limiting factors in determining the rate of glucose utilization in the developing retina.

223. CARBON DIOXIDE REQUIREMENTS OF THE EARLY CHICK EMBRYO.¹ Nelson T. Spratt, Jr., The Johns Hopkins University.

Partial removal of CO_2 (by removal of bicarbonate from the bicarbonate, phosphate buffered-Ringer glucose medium) inhibits differentiation and morphogenesis of the central nervous system but has little, if any, effect on heart formation in the explanted blastoderm. More complete removal of CO_2 (by the additional use of anaerobic jars containing KOH to absorb CO_2) tends to cause more complete degeneration of the entire blastoderm.

The inhibitory effects of CO_2 removal have also been demonstrated by gradually lowering the bicarbonate concentration (from 0.007 to 0.0003 M) in the medium and observing the progressively greater degeneration. These effects are partially reversible by subsequent replacement of CO_2 . At an initially high pH (8-9.5), which favors CO_2 absorption from the air, there is less degeneration during the first and more recovery during the second 24 hour period of explantation than when the initial pH of the medium is lower (7-8). The optimal pH range in the presence of bicarbonate is about 7.3-9.5.

Experiments in progress indicate that the effects of bicarbonate (CO_2) deficiency can be partially alleviated by addition of certain amino acids to the medium. Glutamic acid, alone, is apparently most effective. α -Ketoglutaric acid, a combination of lysine, threonine and methionine, and a combination of arginine, threonine and cystine are less effective. However, none of these amino acids is as efficient as bicarbonate or CO_2 itself in preventing degeneration and supporting further development. It is possible that CO_2 may be as fundamental a requirement of the early embryo as is oxygen.

¹ Aided by a grant from National Institute of Health, U. S. Public Health Service.

224. PURIFICATION OF FIREFLY LUCIFERIN. William D. McElroy and Bernard L. Strehler, The Johns Hopkins University.

A heat stable, dialysable factor from *Photinus pyralis* luminous organs has been isolated by MgO-celite column and paper chromatography, solvent partition and silver precipitation. This factor greatly increases the luminescence of a dilute enzyme preparation which contains excess adenosine triphosphate and MnSO_4 . The active principle is soluble in water, methyl, ethyl, propyl, butyl, amyl and isoamyl alcohol, acetone and ethyl acetate. It is relatively insoluble in benzene, petroleum ether, ethyl ether and CCl_4 . Aqueous solutions of the material are stable to heat (100°C . for 30 minutes) and aeration (18 hours at 27°C .) but its activity is destroyed by strong acids and bases, drying at low temperatures and oxidizing agents. In the acid range the compound has a blue fluorescence and in the basic range a yellow-green fluorescence. The ultraviolet absorption spectrum in isobutyl alcohol is characterized by a maximum at 275 mu, 282 mu and 300 mu, while in phosphate buffer the following maxima are apparent at various pH's: pH 2.2 (275, 281 mu); pH 7.2 (283, 291 mu); pH 11.3 (293 mu). Both *Photinus pyralis* and *Photurus pennsylvanica* contain this material in their luminous organs but not in the remainder of their bodies. The former also contains a variety of other blue-violet fluorescing compounds.

225. REACTION OF PLASMA CELLS IN THE SPLEENS OF RATS FED PROTEIN DEFICIENT DIETS. Paul G. Roofe and Jerry W. Brown, University of Kansas.

Two groups of albino rats were fed diets deficient in protein. One diet contained no protein (0%) and the other diet contained 2% of protein. A third group of rats received a normal or 18% protein diet. At the end of 8 weeks a part of each group were sacrificed and their hemopoietic organs removed. The remaining rats were then placed on the 18% protein diet in order to study recovery processes.

Plasma cells were found only in two areas, the hemopoietic area of the splenic cord and the lymphatic area of the cord. In the hemopoietic area the plasma

cells numbered 39,000/cu mm in the 18% or normal rats. In the 2% animals no plasma cells were found, while in the 0% rats 6,500 cells/cu mm were present. In this area of the 2% and 0% rats after recovery the plasma cells had increased to 34,000 and 55,000 cells/cu mm respectively.

In the lymphatic area of the splenic cord the normal level of plasma cells was 250,000 cells/cu mm. These cells dropped to a population of 11,000 in the 2% rats and to 35,000/cu mm in the 0% rats. After the deficient rats were placed on the normal diet the plasma cells rose to 120,000 cells/cu mm in the former 2% rats and to 200,000 cells/cu mm in the former 0% rats.

226. THE ALKALINE PHOSPHATASE ACTIVITIES OF SUBCELLULAR PARTICULATES.¹

Frances Green Kallman and M. J. Kopac, Department of Biology, Washington Square College of Arts and Science, New York University.

Subcellular particulates were obtained by centrifugal fractionation of homogenates prepared from the intestinal mucosa of rabbits. The scraped mucosa (1 gm) was thoroughly ground (TenBroeck glass grinder) in a cold salt solution (4 ml) consisting of KCl—0.042 M, NaCl—0.0017 M, CaCl₂—0.00043 M. The resulting crude homogenate was refined by several cycles of low speed centrifugation. The refined homogenate was re-centrifuged at various speeds to yield three fractions, e.g., large particulates (1300 G at 40 min.), submicroscopic particulates (18,000 G at 90 min.), and supernatant (from 18,000 G fraction). All processes were carried out in a cold room maintained at 3 to 5°C.

The enzyme (suspensions of particulates, or supernatant) and the substrate (pH 9.4, buffered with Veronal) were incubated at 37°C., and the amount of phosphate released after 15 and 30 minutes' incubation was measured colorimetrically by means of the Soyenkoff-Quinaldine Red method. Essentially all phosphatase activity for the several substrates tested was present in the large and submicroscopic particulate fractions.

With alpha-glycerophosphate and beta-glycerophosphate, separately or mixed, the highest activity (phosphate released/mg N) was found in the submicroscopic fraction. With fructose-1,6-diphosphate the highest activity was found in the large particulate fraction. Phlorhizin inhibited the "beta-glycerophosphatase" activity only slightly, while the "alpha-glycerophosphatase" activity was greatly inhibited in both particulate fractions. On the other hand, the inhibition by phlorhizin was slight when the substrate was a mixture of alpha- and beta-glycerophosphate.

In several instances, l-histidine inhibited the "beta-glycerophosphatase" activity of the submicroscopic particulates more so than of the large particulates.

These and other results support the conclusion that there are many phosphatases, presumably substrate specific, and that these may be differentially localized in two major particulate fractions.

¹ These investigations were supported by Grant C-843, National Cancer Institute, U. S. Public Health Service.

227. THE ACTION OF CATIONS ON THE PROTOPLASM OF AMOEBA PROTEUS AND PELOMYXA CAROLINENSIS.¹ Robert Kassel and M. J. Kopac, Department of Biology, Washington Square College of Arts and Science, New York University.

The present investigation is a search for an intracytoplasmic physiologic medium to be used for homogenization of cells and subsequent fractionation and isolation of subcellular particulates. The solutions were injected directly into the cytoplasm of the ameboid organisms, using Chambers' microinjection procedures. Micro-surface chemical methods were also used. Evidences of injury and recovery were based on the following criteria: (1) changes in rate or type of streaming, (2) sol—gel reactions, (3) the visible condition of the cytoplasmic constituents, and (4) surface denaturation of the cytoplasmic proteins.

The injection of sucrose solutions, frequently used as homogenizing media, induced anomalous sol—gel reactions. Injected portions of the ameba frequently coagulated and subsequently disintegrated. In others, the coagulated masses persisted unchanged for periods of several days. These and other results raise a question as to the value of sucrose as an extraction medium for cytoplasmic fractionation.

Manganese chloride, at appropriate concentrations, generally antagonized the action of calcium chloride by promoting solution. A $(Na + K)/Ca$ ratio of about 40 approaches an optimum balance. Furthermore, the concentration of potassium needs to be about three-fold higher than sodium. To a limited extent, calcium can be replaced by strontium and potassium by rubidium.

One of the most "indifferent" of salt solutions to *Amoeba* and *Pelomyxa* cytoplasm as determined by microinjection is the following mixture: KCl—0.042 M, NaCl—0.017 M, $CaCl_2$ —0.0015 M, $MnCl_2$ —0.001 M. Mixtures lacking in manganese or containing significantly lower concentrations of calcium were clearly inferior.

In spite of the indifferent action of these mixtures on ameboid cytoplasm, when injected in moderate amounts, the surface chemical tests indicated that little or no protection was given to the cytoplasmic proteins. Cytoplasmic proteins released by tearing the cells were highly susceptible to surface denaturation at oil/water interfaces. The next step is to fortify the salt mixtures with substances that may function as anti-denaturing or protecting agents. Such experiments are in progress.

¹ These investigations were supported by Grant C-843, National Cancer Institute, U. S. Public Health Service.

228. TEMPERATURE-PRESSURE EXPERIMENTS ON CELL DIVISION. Douglas Marsland, New York University and the Marine Biological Laboratory.

The level of pressure which is just sufficient to inhibit the first cleavage furrows in sea urchin (*Arbacia punctulata*) eggs and to produce a retreat of the furrows after cleavage has started varies linearly with temperature in the range (10° and 30°C.) in which division will occur. Starting at 10°C., where 3,000 lbs./in.² is adequate to block division, each increment of 5°C. elevates the minimum pressure level by 1,000 lbs./in.², up to 30°C., where it requires 7,000 lbs to stop

cleavage. Also it has been found that the gel structure of the cortical protoplasm of the egg becomes firmer with increasing temperature, as measured by the centrifugal displacement of the cortical pigment granules at various temperatures. In fact, all the data support the hypothesis that the energy available for the furrowing process is determined by the state of gelation of the cortex of the egg and that this gel is of a type that sets more firmly with increasing temperature but tends to undergo solation with increasing pressure.

Cytology

229. EFFECT OF THYMONUCLEODEPOLYMERASE AND ACID HYDROLYSIS ON METHYL GREEN STAINING OF CHROMATIN. Cecile Leuchtenberger, Marion Himes and Arthur W. Pollister, Columbia University.

From analogy with in vitro experiments it has been inferred that the stainability of chromatin with methyl green in Unna's alcohol-phenol-glycerin mixture is dependent upon the desoxypentose nucleic acid (DNA) being in a particular physical state which for convenience has been called highly polymerized. Furthermore, it has been pointed out that, since the Feulgen reaction remains unaffected by an alteration of the DNA by hot water which is sufficient to abolish completely the affinity of the chromatin for methyl green, a quantitative estimation of the ratio between these two stains in cytological preparations can be used to detect deviations from the normal state of the DNA under experimental and pathological conditions (Pollister and Leuchtenberger, *Proc. Nat. Acad. Sci.*, 35: 111-116). Further experiments show that this so-called depolymerization of the DNA to a condition where it is methyl green negative can also be brought about by pretreatment of sections with either hydrolysis in normal HCl or hot 5% trichloroacetic acid and also by digestion with crystalline desoxyribonuclease (thymonucleodepolymerase). Under certain conditions of pH and temperature, the digestion with the nuclease can be controlled to bring about complete loss of methyl green stainability with but slight reduction of the Feulgen reaction. It is not contended that the reduction of methyl green staining by these different treatments necessarily indicates identical changes in the DNA. These experiments confirm the previous conclusion as to the significance of the methyl green reaction as an indicator of physical change in the nucleic acid.

230. THE INSEMINATION OF AMPHIBIAN BODY CAVITY AND OVIDUCAL EGGS. Henry W. Aplington, Jr., Muhlenberg College.

In *Rana pipiens* and other anura studied, oviducal eggs may develop after exposure to sperm suspension while body cavity eggs do not.

It is suggested, on the basis of the following observations, that the egg undergoes in the oviduct a physiological conditioning which is largely independent of the stage of chromosomal maturation: (1) Oviducal eggs are monospermic, body cavity eggs, as shown by the Feulgen technique, admit a number of spermatozoa; (2) in the upper oviduct, eggs are often in the same stage of chromosomal maturation as body cavity eggs; (3) eggs forced to remain in the body cavity form the second polar spindle, a stage usually attained in the oviduct, but when

penetrated by spermatozoa exhibit degenerative changes characteristic of eggs inseminated just after ovulation; (4) of one clutch of pipiens eggs stripped and fertilized a number were fixed at fertilization for later study, the remainder developing normally. The former were uniformly in metaphase to anaphase of the first polar spindle, tetrads being present. Here, eggs in an earlier stage of maturation than many body cavity eggs which do not develop when inseminated, did so after passing through the oviduct.

The conditioning effect of the oviduct is not species specific between *Bufo fowleri* and *Rana pipiens*. Fowleri body cavity eggs transferred by pipette to the body cavity of a previously ovariectomized frog pass through the oviducts and develop on insemination with toad sperm. Reciprocally, pipiens eggs surrounded with fowleri jelly and exposed to pipiens sperm will develop normally.

231. THE EFFECTS OF CARBAMATES ON ONION ROOTS. Verne van Breemen, State University of Iowa. (Introduced by Harold W. Beams.)

This investigation considered the effects of carbamates on onion root tips, in respect to growth, cell division, and viscosity changes within the cells. The following series of carbamate solutions (in water) was used: ethyl carbamate, 0.1 M, 0.2 M, 0.3 M; n-butyl, 0.01 M, 0.0214 M, 0.03 M; isoamyl, 0.004 M, 0.008 M, 0.019 M; ethyl phenyl, 0.0006 M, 0.002 M, 0.006 M.

Changes in length of the roots before, during and after treatment were measured. Treatment caused slowing and eventual cessation of growth (occurring at 60 hours in 0.1 M ethyl carbamate, 35 hours in 0.2 M solution, 11 hours in 0.3 M solution); from this point, the roots began to shrink, the cells to separate, the nuclei to become pyknotic. If the onions were replaced in water within 85 hours (total treatment) from the 0.1 M solution, within 55 hours from the 0.2 M, and within 12.5 hours from the 0.3 M, the roots usually regained normal growth.

During treatment, before the stoppage of growth, mitotic figures, though less numerous, could be found in all stages, mostly in metaphase and anaphase. When growth was blocked, mitotic figures were absent.

Centrifugation of untreated and treated roots at 45,000 times gravity for two minutes showed that the nuclei of the normal cells were moved centrifugally, while the treated cells, being more viscid, were less responsive to centrifugation. Slowly growing treated roots were less affected by centrifugation than were normal roots.

232. LESION INDUCTION IN THE CHORIOALLANTOIS BY SUSPENSIONS OF ROUS SARCOMA, TETRAHYMENA GELEII, AND VARIOUS FRACTIONS OF THESE CELLS. R. A. Fennell, Michigan State College.

Suspensions of Rous sarcoma and alcoholic extractives of these cells induced lesions. Likewise, suspensions of aged Tetrahymena, and also alcoholic fractions of alcohol-chloroform extracts of Tetrahymena, after evaporation and resuspension in normal saline induced lesions in the chorioallantois. Aged specimens of Tetrahymena, after fixation and subjection to the Schiff reagent, showed some specimens with black granules within the nucleus and other with granules in the

cytoplasm. Granules reacted with the reagent in 12-72 hours subsequent to hydrolyzation in normal HCl for 4-20 minutes. Black granules were never demonstrated either in the cytoplasm or nucleus of young organisms. Further, aged specimens exhibited an abundance of fat and glycogen. Cytopathological analyses of lesions will be reported elsewhere.

233. HETEROPLOIDY IN A "NATURAL POPULATION" OF *AMBLYSTOMA PUNCTATUM*.¹
Donald P. Costello and Catherine Henley, University of North Carolina.

Utilizing the tail-tip method described by Fankhauser, a systematic study has been made of *Amblystoma punctatum* larvae, to ascertain the incidence of heteroploidy and abnormalities of mitosis. These larvae were collected at various stages of development near Chapel Hill, and were raised in the laboratory. Both "first" and regenerated tail-tips have been studied cytologically, but only the data from a sample of 231 regenerated tips are included here. Ninety-five larvae were collected from water at 18°C., and maintained at laboratory temperature after removal from the jelly mass. Of these, 47 were normal diploids, 19 had nuclei of variable size and/or variations in nucleolar number, 22 had abnormal mitoses, and 7 were chromosomal mosaics. In a group of 60 larvae collected and maintained as above, but not removed from the jelly, 26 were normal diploids, 4 had variations in nuclear size and/or nucleolar number, 24 had abnormal mitoses, and 6 were chromosomal mosaics. Seventy-six larvae (44 of which appeared to have been frozen) were collected from very cold water, removed from the jelly, and maintained at 11°C. Of these, 36 were normal diploids, 17 had variations in nuclear size, 20 had abnormal mitoses and three were chromosomal mosaics. Two larvae of this group had three nucleoli in every nucleus, although chromosome counts showed the diploid number of chromosomes (28) to be present. Seven tail-tips had exceptionally clear multipolar figures. The types of mosaics found included haploid/diploid, hypodiploid/diploid, diploid/triploid, and diploid/tetraploid.

¹ Aided by grants from the American Philosophical Society and the Carnegie Research Fund of the University of North Carolina.

234. THE FINER STRUCTURE OF THE RESTING CHROMOSOME. William Hovanitz, University of San Francisco.

Fragmentation or "dissociation" of isolated chromosomes and study with the electron microscope has led to an extended interpretation of their helical or spiral structure. The normal whole chromosome of about 3000 A in diameter is composed of two strands each having a diameter of about 1500 A. Each of these is spiral with a distance between coils of 3000 A. The two are closely appressed but do not coil around one another, the result being a solid-appearing strand of 3000 A. Each 1500 A strand is further composed of two strands of about half the diameter, closely appressed in the same way. Subdivisions of the chromosomes indicate strands of 100 and possibly 50 A in diameter as minimum sizes having a helical structure.

Ecology

235. BODY TEMPERATURE OF LEACH'S PETREL. G. Edgar Folk, Jr., Bowdoin College.

The Leach's Petrel lays one egg a year in an underground burrow, and it has an incubation period at least 40 days long. Each nesting adult spends three or 4 days at sea, and then returns to relieve its incubating mate. Many petrels live 9 to 10 years or more. Body temperature measurements of petrels are of particular interest because of this longevity, unusual nesting behavior and incubation period. Preliminary measurements were made in June, on 12 petrels at the Bowdoin Scientific Station, Kent's Island. Forty-two cloacal temperatures were taken, using a standard procedure. Maximum-minimum air temperatures were recorded in and near nesting burrows. Standard body temperatures obtained in the laboratory averaged 39.1°C. (extremes, 7 birds, 38.3°–39.9°C.) for birds conditioned at 13.3°–16.8°C. Maximum body temperature due to muscular exercise averaged 39.4°C. (extremes, 6 birds, 38.5°–40.1°C.). Markedly different results were obtained in field studies on all birds which were incubating eggs. Readings of 37.9°, 37.5°, and 35.8°C. were unaffected by 5 minutes of forced activity of the birds. Four determinations of incubation temperature by a mercury thermometer under the bird and against the egg, averaged 17.8°C. (extremes 15.5°–19.2°C.). Air temperatures in burrows varied from 6.2°–11.1°C.

The laboratory results indicate that the standard temperature of the petrel is lower and the effect of exercise less, than in the species studied by Baldwin and Kendeigh ('32). The low body and nest temperatures of incubating petrels suggest a semi-torpid condition in these birds, such as reported in other species by McAtee ('47). Such a state of semi-torpidity would be compatible with both the fasting of the incubating adult, and the long incubation period of this species.

236. FURTHER STUDY OF THE PSELAPHID BEETLE POPULATION OF AN ILLINOIS PRAIRIE. Orlando Park, Stanley Auerbach and Marie Wilson, Northwestern University.

The present report continues the study of pselaphid beetles from Peacock Prairie, but with the emphasis shifted to an examination of the population from a quantitative and seasonal point of view. This is a 10 acre tract that has not been grazed or cultivated, near Evanston, Illinois. Since November 1947 relatively regular weekly samples of prairie soil and sod have been collected. Each sample covered 0.08 m², to a depth of three inches, and averaged 5000 gm in weight. Each sample was weighed, the pselaphids were then extracted by the Berlese method, and then the dry sample weighed to determine free water content. Population density was worked out on an average square meter and average acre basis. The pselaphid population consisted of 7 species. It reached a peak during late spring—early summer (April, May, June), when the beetles averaged about 70 per square meter, and reached a low during January and February of about 3 per square meter. Evidence at hand, including the frequency of postpupal "callows," suggests two

generations a year: an overwintering adult population migrates vertically into the deeper soil layers, emerges to mate and produce the first generation in the spring; spring population mates and produces a second generation that appears in autumn; this autumn generation overwinters to complete the cycle. Soil moisture appears to be an important factor in population distribution. This study is being continued.

237. ON CULTIVATION OF LARVAE OF *OSTREA LURIDA*. H. C. Davis, U.S. Department of the Interior, Fish and Wildlife Service. (Introduced by V. L. Loosanoff.)

Several cultures of the larvae of *Ostrea lurida* have been grown to metamorphosis using the method developed at Milford Laboratory for rearing lamellibranch larvae. To collect the veligers of this larviparous species, as they are released by the parents, gonad maturation and spawning of the adult oysters were induced in aquaria of warm, aerated, filtered sea water, which was changed daily through fine screens. The temperature was maintained between 15.0 and 18.0°C. throughout maturation, spawning and incubation. A mixed food culture of green phytoplankton, consisting chiefly of a species of *Chlorella*, was added daily.

A light spawning was noted on April 12, and a heavier one on April 16. A few larvae were recovered on April 20-21 and a much larger number on April 23-24. This would indicate that, under the experimental conditions, the incubation period, i.e., time from spawning to swarming, varied from 7 to 9 days.

Although most of the larvae at the time of release measured 169 to 185 μ , sometimes larvae were released while still only 158 μ in length. In the cultures kept at 19.0-22.0°C. growth appeared to be normal and the larvae metamorphosed when about 300 μ in length. The free-swimming period in these cultures varied, as the result of several factors, from 10 to 23 days with a mean of 16 days. Cultures kept at temperatures ranging from 16.0 to 18.5°C. showed considerable growth. Many individuals reached the eyed larval stage of 275-290 μ , but no metamorphosis took place although some larvae lived for 30 days. At temperatures ranging between 14.0 and 16.0°C. some of the larvae lived for 20 days but grew only to a size of 220 μ .

After metamorphosis growth of the spat of *O. lurida* was somewhat more rapid than that of the spat of *O. virginica*, which metamorphosed at the same time and were kept under the same conditions. Some of the *O. lurida* attained a length of 3.5 to 3.7 cm by the 70th day after metamorphosis.

238. VERTICAL DISTRIBUTION OF OYSTER LARVAE OF DIFFERENT AGES DURING THE TIDAL CYCLE. V. L. Loosanoff, U.S. Department of the Interior, Fish and Wildlife Service.

These observations were carried on in Long Island Sound and Milford Harbor for several summers. Samples were collected at hourly intervals throughout the tidal cycles at the bottom, middle layer, and near the surface. Oyster larvae were present at all levels. They were often least common near the low water stage, while at and near high water they became more numerous. In several instances the number of larvae was greatest midway between the high and low water stages, when the

tidal current was near or at its maximum velocity. In general, no relation was found between the stratification of larvae and the tidal stages and there was no evidence that larvae in advanced stages of development, such as, early or late umbo, were more common near the bottom. These observations show that in Long Island Sound oyster larvae do not descend to the bottom during periods of rapid tidal flow and are, as a result, widely dispersed by the tidal currents.

239. GONAD DEVELOPMENT AND SPAWNING OF OYSTERS AT SEVERAL CONSTANT TEMPERATURES. V. L. Loosanoff and H. C. Davis, U. S. Department of the Interior, Fish and Wildlife Service.

These experiments were conducted on oysters taken from Long Island Sound in winter, when their gonads were in the undeveloped stage. The oysters were placed in laboratory trays with running water the temperature of which was maintained within $\pm 1^\circ$ at 10.0, 15.0, 20.0, 25.0 or 30.0°C. The progress of gonad development was ascertained at 5-day intervals. The purpose of the experiment was to determine the day-degrees needed for the development of mature gametes.

Even the most advanced oysters kept at 10.0°C. showed only a slight development of gonads after 35 days' exposure. The follicles remained contracted; there was almost no yolk deposition in the oocytes, and in the males secondary spermatocytes were present but no spermatids were formed.

At 15.0°C. the most advanced males contained a few sperm on the 10th day. On the 15th day sperm was used in successful fertilization of the eggs. Almost all the males had active sperm on the 20th day. Fertilizable eggs were found in some females on the 30th day. After 35 days spawning of a few females could be induced by high temperature. After 60 days the oysters were releasing small quantities of spawn, and on the 90th day mass spawning was induced at 15.8°C. by the addition of a suspension of sperm and eggs.

At 20.0°C. males with ripe sperm were found on the 10th day. Fertilizable eggs were obtained on the 15th day. Spawning was induced on the 20th day.

At 25.0°C. rapid spermatogenesis was observed during the first 5 days. Unprovoked mass spawning occurred on February 25, the 9th day of exposure.

At 30.0°C. ripe sperm was formed three days after the hibernating oysters were taken from their winter environment and placed at this temperature. On the 5th day several females had small numbers of physiologically mature fertilizable eggs.

Embryology

240. THE EFFECT OF ANTUITRIN S ON THE OVARIES OF IMMATURE HAMSTERS (*CRICETUS AURATUS*). F. V. Rollins and S. Milton Nabrit, Atlanta University.

Three cubic centimeters of Antuitrin S (Parke, Davis) were daily injected subcutaneously in the abdominal region of female hamsters from the 21st through the 26th day after birth.

After Bouin's killing and Erlich's haemotoxylin staining the ovaries were compared with uninjected controls.

Follicular number was approximately equal in the controls and the experimental animals of comparable age. A larger number of mature follicles, those with an antrum and the ovum eccentrically located, were found in the injected animals. More follicles with a well loosened granulosa also appeared in the experimental animals.

Corpora lutea were lacking in both experimental and control animals.

It appeared that Antuitrin S when injected at the age 21 days hastened maturity rather than increased follicular number.

241. A HISTOLOGICAL STUDY OF FORELIMB REGENERATION IN *TRITURUS VIRIDESCENS*. Lemuel A. Lewie, Jr., and S. Milton Nabrit, Atlanta University.

A histological study of the regeneration from stumps of both forelimbs after removal of the distal third of the lower arm was made. Decalcified stumps cut in longitudinal serial sections were studied from wound closure to the complete formation of digits.

Dedifferentiation of epidermis, muscle, cartilage and connective tissues formed a regeneration blastema. All kinds of needed tissues appeared to redifferentiate from the blastema. The preblastema period was much longer than that observed by Thorton on *Amblystoma*. The differentiation from the blastema is slower. Dedifferentiation does not appear as pronounced in *Triturus* as in *Amblystoma*. The epidermis appeared to make the greatest initial contribution to the blastema with the other tissues equalizing their contribution in later stages.

242. THE DISTRIBUTION OF PHOSPHORUS³² IN THE EARLY CHICK EMBRYO IN THE PRESENCE OF VITAMIN D. Louis A. Hansborough and Philip Nicholas, Howard University.

Phosphorus³² and vitamin D₃ were injected into the air-chamber of the hen's egg before incubation. Autoradiographs made on lantern slide plates and dental x-rays films of 48- and 72-hour embryos, whole mounts and x-sections, revealed that the phosphorus is readily utilized in these early stages of development, and that phosphorus turn-over increases with the age of the embryo. The pattern of phosphorus distribution follows the pattern of cell distribution, the concentration of phosphorus being highest in the areas where the accumulation of cells is greatest. In the order named, these areas are: nervous system, tail and limb-buds (72-hour embryo), heart, somites, blood vessels, gut and lateral mesoderm. The process is not tissue-specific.

243. LOCALIZATION OF PROSPECTIVE AREAS IN THE BLASTODERM OF THE PEA BEETLE *CALLOSOPRUCHUS MACULATUS* FABR., AS DETERMINED BY ULTRAVIOLET (2537 Å) IRRADIATION INJURY. Alfred Brauer, University of Kentucky and Biology Division, Oak Ridge National Laboratory.

The egg of the pea beetle *Callosopruchus maculatus* was subjected to ultraviolet irradiation in three early developmental periods for the purpose of analyzing: (a) value of cleavage cells in organizing development, and (b) localizing presumptive areas in early blastoderm and embryonic plate stages. For the first

purpose the egg was irradiated entire or in part, and for the second it was subjected to spot irradiation by admitting radiation (2537 Å) of known intensity to definite areas of the blastoderm. The incorporation of the resulting injured areas was followed in the developing embryo. When all or a part of the first cleavage cells are severely injured, development nevertheless proceeds to varying extent and is always organized and integrated. This is interpreted to mean that organization of development is the prerogative of the egg as a whole and the cytoplasmic cortex rather than of the cleavage cells. In the early blastoderm the principal formative areas are localized in cells of the central, ventral blastoderm and extending somewhat anterior and posterior to this center. In the later blastoderm, formative areas are projected anteroposteriorly along, and laterally from the median line. Injury to the suprasuboesophageal ganglion-forming area results in serious inhibition to the more anteriorly situated "head-forming" area as well. Maps of blastoderms showing formative areas have been constructed on the basis of the data.

244. DETERMINATION OF THE RELATIONSHIP BETWEEN THE PRINCIPAL EGG AND LARVAL AXES OF *SACCOGLOSSUS* (*DOLICHOGLOSSUS*) *KOWALEVSKYI*. Arthur L. Colwin and Laura Hunter Colwin. Queens College, Flushing, N. Y.

The living egg of *Saccoglossus kowalevskyi* shows no visible landmarks other than the polar bodies. Various blastomeres were stained with Nile Blue Sulphate and their development followed until the first pair of gill-slits appeared. In 172 cases one of the first two blastomeres was stained. The subsequent larvae showed either the right or the left half stained, the color extending from about the mid-dorsal to the mid-ventral line. Hence the first cleavage plane parallels the antero-posterior axis and the plane of bilateral symmetry of the larva. To determine the relationship of the second cleavage plane, two adjacent blastomeres of the 4-cell stage was stained, each originating from a different one of the first two blastomeres. In 53 cases the larvae showed the color either dorsally, including both of the first gill-slits (which are located dorso-laterally), or ventrally, extending ventro-laterally but not as far as the gill-slits. Hence the second cleavage furrow parallels the antero-posterior axis of the larva, but is perpendicular to the larval plane of bilateral symmetry. The third cleavage results in an upper, animal tier of 4 small cells and a lower, vegetal tier of 4 larger cells. In 45 cases the animal tier was stained and the subsequent larvae showed the anterior region colored. In 40 cases the vegetal tier was stained and the subsequent larvae showed the posterior region colored. Hence the third cleavage plane parallels the dorso-ventral axis of the larva and is perpendicular to the larval antero-posterior axis.

245. THE MODIFICATION OF DEVELOPMENTAL PATTERN IN THE SAND DOLLAR WITH SODIUM AZIDE. Olin Rulon, Northwestern University.

Newly fertilized eggs and 6-hour blastulae of *Dendraster excentricus* were exposed to M/200-M/800 sodium azide (made up in sea water) for periods ranging from 6 to 60 hours. It was found that blastulae were better able to tolerate the agent and showed better recovery on return to sea water than

newly fertilized eggs. With continuous exposure to M/800, beginning at the 1-cell stage, differential tolerance of ectoderm was better, while differential tolerance of ventral ectoderm was better with continuous exposure to the same concentration beginning at the blastula stage. Differential recovery of ventral ectoderm occurred following 6 and 18 hours initial exposure of newly fertilized eggs to M/800. Approximately 90% exogastrulation (with entodermization in the higher concentrations) occurred following 18 hours exposure of newly fertilized eggs to M/400–M/800. Differential recovery of ventral regions occurred following 12 hours exposure of blastulae to M/200. Blastulae exposed for 36 hours to M/200 (followed by return to sea water) developed chiefly into highly entodermized forms with little or no ectodermal differentiation.

Practically all of the modifications caused by azide on this form have been produced by numerous and different agents (lithium, thiourea, pilocarpine, thiocyanate, cyanide, tobacco smoke, crowding, etc.) and may be interpreted as the results of differential inhibition, conditioning, or recovery phenomena in the gradient system of the egg.

246. THE MODIFICATION OF DEVELOPMENTAL PATTERN IN THE SAND DOLLAR WITH THIOUREA. Olin Rulon, Northwestern University.

Newly fertilized eggs and 6-hour blastulae of *Dendroaster excentricus* were placed in solutions of thiourea (100 cc of sea water plus 5.0–20.0 cc of 0.54 M thiourea) for periods ranging from 6 to 60 hours.

Despite the fact that thiourea may be classified as a specific enzyme inhibitor (inhibitor of peroxidase and possibly certain other oxidative enzymes) its effects at critical concentrations and exposure intervals on developmental pattern were quite similar to those produced by numerous and very different agents (lithium, azide, pilocarpine, thiocyanate, cyanide, tobacco smoke, crowding, etc.). Beginning with the newly fertilized eggs continuous exposure to the lower concentrations inhibited oral lobes and anal arms and caused approximately 20% exogastrulation. With the higher concentrations the ectoderm became more inhibited and the size of the evaginated entoderm increased. Modifications caused by exposure to thiourea beginning at the blastula stage were not greatly different from those caused by exposure of the newly fertilized egg. Blastulae, however, were able to tolerate concentrations that were lethal to the uncleaved egg. In the highest concentrations entodermal development as well as ventrodorsality were inhibited although a certain degree of polarity still persisted.

Thiourea was less effective in the production of exogastrulae than sodium azide, possibly because azide is a better inhibitor of cytochrome oxidase activity — an activity that parallels and may be an important correlate of the primary gradient system in this egg.

247. LOCALIZATION OF HEMOGLOBIN-FORMING AREAS IN THE EARLY CHICK BLASTODERM CULTIVATED IN VITRO. George W. Settle, The Johns Hopkins University. (Introduced by Mary E. Rawles.)

The early chick blastoderm (pre-streak to 6 somite stages) was divided by a variety of longitudinal and transverse cuts into a number of pieces. The original position of each piece was accurately recorded by means of camera lucida draw-

ings. Pieces were explanted separately to a medium containing yolk-albumen extract plus saline agar, and incubated at 37.5°C. Explants were checked after 100 hours for hemoglobin formation, and results tabulated in the form of "maps" of the early blastoderm. Two criteria of hemoglobin formation were used: (1) appearance of characteristic red color, and (2) specific staining with patent blue (after Dunn).

Studies of the maps prepared show a progressive change in the distribution of areas with hemoglobin-forming capacity. In pre-streak stages, the hemoglobin-forming area is limited to the posterior half of the area pellucida. Further disposition of these areas, as development proceeds, shows two major trends: (1) in the anterior-posterior axis, regression from a point midway in the long axis of the streak to the posterior border of the area pellucida; and (2) in the lateral axis, movement of the hemoglobin-forming areas first toward the streak, and following this, spreading toward the lateral border of the blastoderm, finally being localized in the area vasculosa.

The striking parallel between the progressive shift in hemoglobin-forming areas of the blastoderm and known morphogenetic movements suggests that these areas have their origin in the epiblast, secondarily undergoing invagination through the streak to their definitive positions.

248. CAN PROSPECTIVE PIGMENT CELLS OF THE CHICK EMBRYO BE IDENTIFIED BY MEANS OF VITAL DYES DURING THE COURSE OF THEIR MIGRATION INTO THE SKIN AND FEATHER GERMS? John W. Saunders, Jr., The Johns Hopkins University and the University of Chicago.

To answer this question, living embryos (various pigmented breeds) of 20 hours to 12 days of incubation were stained *in vitro* for 15 minutes in solutions (1/10,000) of Nile Blue Sulfate or Neutral Red, and examined in isotonic salt solution. Certain cells, intensely stained by this treatment, show a pattern of distribution strikingly similar to that of neural crest cells. In successive embryonic stages, these cells may be traced progressively away from the neural tube and into the limb buds and body wall, attaining the ventral mid-line after approximately 96 hours of incubation. By the 6th day, the prospective dermis is densely populated with the distinctive cells, which then enter the epidermis and synthesize pigment.

Cytological examination of the stained cells shows a closely graded sequence of changes leading to the appearance of a typical melanophore. Tissue isolates containing cells characteristic of the early stages of the sequence, from the flank and leg bud of 33-35 somite Barred Rock donors, produce pigment in grafts to the chorioallantois of White Leghorn hosts. Cells of later stages in the sequence, which differentiate into melanophores in tissue-culture preparations of embryonic skin, are identical to cells found in the barb ridges of stained regenerating feather germs.

Since the distribution of vitally stained cells corresponds to known sources of melanoblasts (neural crest, barb ridges; Dorris, '38; Ris, '41; Foulks, '43;

Rawles, '44) established by grafting techniques, it appears that prospective pigment cells may be identified and the course of their migration followed by means of the vital dyes used.

¹ Work aided by the Abbott Memorial Fund of the University of Chicago, and by the American Cancer Society through the Committee on Growth.

249. THE DEVELOPMENT OF SPINAL GANGLIA FOLLOWING THE AMPUTATION OF HIND LIMB BUDS IN *RANA PIPIENS* LARVAE. James W. Merritt, The State University of Iowa. (Introduced by Jerry J. Kollros.)

Unilateral hind limb amputations were performed on two groups of *Rana pipiens* larvae, at developmental stages III and X. The animals were sacrificed at intervals until metamorphosis, and the hypoplasia in the spinal ganglia of the limb segments was determined by making complete cell counts of the ganglia on both normal and operated sides. Separate counts were made of the large, differentiated cells.

Significant hypoplasias on the operated side were found as early as 6 days after operation; at metamorphosis the decreases in total cell number were 65.2% and 69.5% for the stage III and X groups respectively. The hypoplasia of the large cells developed faster than did that of the total number of cells. The ratio of large cell number to total cell number was significantly lower on the operated side than on the normal side in both groups.

The counts of both large and small cells in the ganglia on the operated side of the older animals showed a significant decrease from similar counts of younger animals in the same group. This indicates an atrophy or degeneration similar to that found in the chick by Levi-Montalcini and Levi. Degenerating nuclei were seen, and a smaller average size of the "large" cells was observed, but not determined quantitatively, in the ganglia on the operated side of the older animals. All of these evidences for degeneration were found to be more pronounced in the animals which were operated upon at stage X.

250. NUTRITIONAL REQUIREMENTS OF THE EARLY CHICK EMBRYO. III. THE METABOLIC BASIS OF MORPHOGENESIS AND DIFFERENTIATION AS REVEALED BY THE USE OF INHIBITORS.¹ Nelson T. Spratt, Jr., The Johns Hopkins University.

In addition to the naturally occurring hexoses and maltose, the explanted blastoderm can also utilize for early morphogenesis and differentiation 10^{-2} – 4×10^{-4} M sodium pyruvate and sodium lactate, but not succinate, fumarate, α -ketoglutarate, citrate, or acetate (each when present as the sole carbon source in a buffered-Ringer solution with or without agar). Development on pyruvate or lactate is, however, inferior to that on the sugars: embryos shrink in size and practically all development ceases 20 hours after explantation. When both glucose and pyruvate (even at a molar concentration ratio of 1:10 respectively)

are present in the medium, the embryos are similar in appearance to those developing on glucose alone and not dwarfed like the pyruvate controls.

Embryos explanted to glucose media containing the metabolic inhibitors, CH_3ICOOH ($5 \times 10^{-5}\text{M}$) and NaF (10^{-2}M) rapidly undergo complete degeneration and disintegration. These effects are reversible by substitution of $2 \times 10^{-2}\text{M}$ pyruvate or lactate for the glucose. This suggests that the pathways of carbohydrate metabolism in the early embryo are probably similar to those in the adult chicken and in many other organisms.

Sufficiently high concentrations of other inhibitors, NaCN , NaN_3 , citrate, malonate, also cause complete degeneration of the explants. At lower inhibitor concentrations (10^{-5}M CH_3ICOOH , 10^{-2}M NaCN , 10^{-4}M NaN_3 , 10^{-3}M malonate, etc.) and the central nervous system degenerates or fails to form, but the heart develops and pulsates. The only exception to this is NaF which causes degeneration of the heart at concentrations ($5 \times 10^{-3}\text{M}$) which do not appreciably affect the central nervous system.

¹ Aided by a grant from National Institute of Health, U. S. Public Health Service.

251. CARBON DIOXIDE REQUIREMENTS OF THE EARLY CHICK EMBRYO.¹ Nelson T. Spratt, Jr., The Johns Hopkins University.

Partial removal of CO_2 (by removal of bicarbonate from the bicarbonate, phosphate buffered-Ringer glucose medium) inhibits differentiation and morphogenesis of the central nervous system but has little, if any, effect on heart formation in the explanted blastoderm. More complete removal of CO_2 (by the additional use of anaerobic jars containing KOH to absorb CO_2) tends to cause more complete degeneration of the entire blastoderm.

The inhibitory effects of CO_2 removal have also been demonstrated by gradually lowering the bicarbonate concentration (from 0.007 to 0.0003 M) in the medium and observing the progressively greater degeneration. These effects are partially reversible by subsequent replacement of CO_2 . At an initially high pH (8-9.5), which favors CO_2 absorption from the air, there is less degeneration during the first and more recovery during the second 24-hour period of explanation than when the initial pH of the medium is lower (7-8). The optimal pH range in the presence of bicarbonate is about 7.3-9.5.

Experiments in progress indicate that the effects of bicarbonate (CO_2) deficiency can be partially alleviated by addition of certain amino acids to the medium. Glutamic acid, alone, is apparently most effective. α -Ketoglutaric acid, a combination of lysine, threonine and methionine, and a combination of arginine, threonine and cystine are less effective. However, none of these amino acids is as efficient as bicarbonate or CO_2 itself in preventing degeneration and supporting further development. It is possible that CO_2 may be as fundamental a requirement of the early embryo as is oxygen.

¹ Aided by a grant from National Institute of Health, U. S. Public Health Service.

252. THE CUMULATIVE EFFECT OF OXYGEN-PRESSURE IN THE BLOCKING OF GASTRULATION IN THE EMBRYO OF *RANA PIPIENS*. Olin E. Nelsen, University of Pennsylvania.

It is possible to block the gastrulative processes of the *Rana pipiens* embryo utilizing oxygen at 45-46 lbs. pressure added to the normal air in a pressure chamber. Recently fertilized and various ages of developing blastulae and gastrulae were used. A temperature of 8-10°C. was maintained during the exposure period for control and experimental embryos. The controls consisted of: (a) developing eggs kept under ordinary air pressure conditions, and (b) eggs exposed in a chamber with the air pressure increased to 45-46 lbs. Developing eggs under exposure were placed either: (a) in low stender dishes covered by $\frac{1}{8}$ to $\frac{1}{4}$ inch of water, or (b) in a glass cylinder suspended from the surface in a string-like mass to a depth of $1\frac{1}{2}$ inches.

These results were obtained:

- a. Recently fertilized eggs exposed in flat stenders for 21-24 hours and removed from the chamber in the 4 or 8 cell condition experience complete blockage at the end of the blastular period.
- b. Similar experiments utilizing the cylinder method produced complete blockage in the embryos near the surface, complete escape in the lower portions of the egg-string, and various degrees of blockage in the intermediate region.
- c. Developing eggs become increasingly resistant to the blocking effect as blastulation progresses; those exposed toward the end of the blastular state are not affected.
- d. An exposure of 8 hours or less permits total escape from the blocking-effects.
- e. Increased air pressure alone does not produce blockage.

253. CONNECTIONS BETWEEN CEREBRAL CELLS AND THIGH MUSCLES IN THEIR SIMULTANEOUS BREPHOPLASTIC INTRAOCULAR TRANSPLANTATION IN THE MOUSE. Raoul Michel May, University of Paris.

There were implanted simultaneously in the anterior chamber of the eye of 110 adult albino mice a fragment of thigh muscle and fragments of cerebral matter from the same newborn mouse.

Seven days after the double implantation there were present direct connections between the cerebral and the muscular tissues, the cerebral fibers showing intimate relation with the striated muscle fibers. Entire bundles of nervous expansions deviated towards the muscle masses; individual nerve fibers left these bundles to unite with the muscle fibers.

Sixteen days after the transplantation the nerve fibers were present on the muscle fibers, in number and in their courses, very much as they appear in a normal newborn thigh muscle. Their endings, however, were very elementary, without nuclei in a plate, but with a simple enlargement or flattening of the striated fiber.

Twenty-seven days after the graft the muscle had the aspect of a myomatous mass which was richly innervated.

These results show that muscles from a newborn mouse are able to attract the axons of cerebral cells and to bring about the formation of simple endings. Like degenerated nerves (May 1945), these striated muscle fibers cause regeneration of the fibers of very young cerebral cells of the mouse.

254. DEVELOPMENT OF PORTIONS OF 72- AND 96-HOUR AVIAN OTOCYSTS IN CHORIO-ALLANTOIC GRAFTS. Edythe M. Nagler and Hiram J. Evans, Syracuse University.

Otocysts of 72- and 96-hour chick embryos were divided into dorsal and ventral or medial and lateral halves. The transverse cut was made so as to separate the pars superior from the pars inferior labyrinthi. Each half of the otocyst was wrapped in a mantle of splanchnopleure and transplanted to the chorio-allantois of a 9-day host embryo. The control age of the grafts is from 10 to 12 days.

Good histological differentiation was obtained while morphological differentiation was negligible. In general, the development of the half ears was comparable to development of isolated entire 72- and 96-hour otocysts. The dorsal, ventral and medial halves of the ear gave rise to a complete semicircular canal or projections interpreted as canal rudiments, utriculus, sacculus, lagena and endolymphatic rudiment. Sensory areas of both crista and macula type could be identified. Grafts of the lateral half of the otocyst consistently lacked an endolymphatic portion, but other parts of the inner ear, including the sensory areas, could be identified.

A few transplants of quarters of the 96-hour otocyst have been made. All grafts tended to form only cystic vesicles. Grafts of the anterior-dorsal quarter were the only ones which showed differentiation of sensory areas.

255. THE EFFECT OF SODIUM SUCCINATE ON THE DEVELOPMENT OF SUCCINIC DEHYDROGENASE IN *AMBLYSTOMA PUNCTATUM*.¹ E. J. Boell, Osborn Zoological Laboratory, Yale University.

Embryos of *Amblystoma punctatum* were reared in solutions of sodium succinate and in saline solutions of corresponding molarity. The embryos were subjected to treatment at stages 19-26, and the duration of treatment varied from several days to several weeks. At intervals, the succinic oxidase activity of morphologically identical embryos from the treated and control groups was determined.

In control embryos, the development of succinic oxidase between stages 15 and 46 follows an exponential course. The rate of increase, or the value of K in the equation $X = ae^{Kt}$, at 25°C. is 0.006. In the treated embryos, the increase of succinic oxidase is likewise exponential, but the rate of increase is considerably greater. Apparently embryos exposed to succinate develop normally with respect to external morphological features, but certain physiological peculiarities have been noted.

In order to determine whether the effect of sodium succinate is specifically on succinic dehydrogenase or on some other component of the succinic oxidase system, determinations of succinic dehydrogenase were made on embryo homogen-

ates by the methods of Quastel and Wheatley. The results of these experiments show that succinic dehydrogenase is apparently induced to undergo accelerated development in the presence of sodium succinate. The similarity of these findings to the phenomenon of enzyme adaptation in micro-organisms is at once apparent; their significance lies in the demonstration of the biochemical lability of embryonic tissues and in the possible morphogenetic effects of biochemical alterations.

¹Supported in part by a grant from The American Cancer Society through the NRC Committee on Growth.

256. DEVELOPMENT OF CHOLINESTERASE IN THE CENTRAL NERVOUS SYSTEM OF *AMBLYSTOMA PUNCTATUM*.¹ E. J. Boell and S. C. Shen, Osborn Zoological Laboratory, Yale University.

Normal differentiation of cholinesterase in various regions of the developing central nervous system was studied in the larvae of *Amblystoma punctatum*. Cholinesterase first appears in detectable amounts (as measured by the ultra-sensitive cartesian diver apparatus) in the spinal cord. During the period when the embryo is capable of making only myogenic responses to tactile stimuli, the enzyme is undetectable in the cord, but its appearance in the cord coincides exactly with the ability of the embryo to respond neurogenically (at stages 35-36). At this time no cholinesterase can be detected in hind-, mid-, and fore-brain. During subsequent development, however, the enzyme appears in these regions of the C.N.S. sequentially from posterior to anterior. In the hind-brain, it is first demonstrable at stage 37; in the mid-brain, at stage 39; and in the fore-brain, at stage 42. In each region the course of increase of enzyme activity follows an approximately sigmoid curve between the time of its first appearance and the early feeding stages.

At all stages studied, the rate of increase and the ultimate level of enzyme activity is highest in the cord and is progressively lower in hind-brain, mid-brain and fore-brain respectively. The data suggest that the increase in cholinesterase activity is apparently associated with functional differentiation and not simply with increase in size of the various regions of the central nervous system.

¹Supported in part by a grant from The American Cancer Society through the NRC Committee on Growth.

257. PITUITARY STUDIES IN THE GOLDEN HAMSTER, *CRICETUS AURATUS*. I. NORMAL DEVELOPMENT AND CELLULAR DIFFERENTIATION. Valeria M. Krol, The University of Chicago. (Introduced by Carl R. Moore.)

Fifty-five hamster pituitaries of both sexes were utilized for this study. The ages ranged from 11 days 19 hours of gestation to over one year of age but emphasis on cellular detail continue only to the 26th day post-partum. Other detailed observations will be reported later along with experimental studies now under way.

The hamster pituitary before birth consists of 4 lobes without cellular differentiation. The anatomy and histology of the normal adult hamster conforms to that of other mammals.

Cytoplasmic elaboration of the anterior lobe originates caudally and progresses rostrally. By the 6th day, most of the acidophils stain blue with modified Mallory's technique, though a few are found to stain red. In addition to these, are the transitional acidophils which show three colors: deep red granules surrounding the nucleus, then the bluish-purple outside of these, followed by the blue granules located at the periphery. There is no intermingling of the different colored granules. Apparently, the differentiating influence radiates outward from the nucleus. These dual staining cells are noted in both sexes up to the 26th day. In the succeeding stages, the blue tinctorial acidophils become inversely proportional in numbers to the red staining type. The basophils are scarce in the 10 day old animals; show a gradual increase by the 16th day. The chromophiles increase rapidly between the 16th and 26th day. Mitotic activity is twice as high in numbers for the female as for the male on the 6th, 16th and 26th day but it is 7 times greater by the 10th day.

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258. HISTOCHEMICAL EVIDENCE OF EARLY DIFFERENTIATION OF THE SUPRARENAL GLAND OF THE CHICKEN. Alden B. Dawson, Harvard University.

Silver impregnation (Bodian's protargol method) has been used to follow the early history of the medullary tissue, since the differentiating cells are more readily identified by their blackened granules than by the chromaffin reaction employed by earlier investigators. Medullary cells within the suprarenal complex may be first recognized late in the 5th day or early in the 6th day of incubation, only slightly earlier than previously reported. Histochemical evidence of differentiation of the elements of the cortical cords (sudanophilia, positive Schiff and Schultz responses, and birefringence in formalin-fixed, frozen sections) is usually obtained regionally 24 hours later but such positive reactions are not uniformly and consistently present in all the cords until 11 to 12 days. At this time the cortical tissue is clearly outlined by all methods employed. These cytological indications of early functional capacity of medullary and cortical components of the chick suprarenal gland are in accord with other observations on the early differentiation of the hypophysis, thyroid gland, and pancreatic islands.

259. PARTIAL MAINTENANCE OF THE ADRENAL CORTEX BY INTRAOCULAR GRAFTS OF ANTERIOR PITUITARY TISSUE IN THE ADULT GUINEA PIG. Malvina Schweizer and Margaret E. Long, Washington Square College.

Adult (male and female) guinea pigs were implanted in the anterior chamber of the eye with the anterior pituitary from a donor of the same sex. One to 4 weeks after implantation the animals were hypophysectomized and sacrificed at intervals of three to 10 weeks after hypophysectomy. Unimplanted guinea pigs, sacrificed two, three, 4, and 6 weeks after hypophysectomy, served as controls. Alterations in

relative adrenal weight, white blood cell count, glomerulosa width and distribution of sudanophilic substance in the glomerulosa and fasciculata were studied. The white blood cell counts were quite stable in control and experimental animals. Changes in the relative adrenal weight were indicative only when they were in the middle or upper end of the normal range. At the low end of normal range they could not be used alone as a criterion to distinguish between partially maintained and non-maintained adrenals. The widening of the glomerulosa, characteristic of hypophysectomy, occurred to the same extent in all operated animals whether an implant was present or not. Lipid droplets stained by sudan black B in frozen sections gradually disappear from the glomerulosa after hypophysectomy. The presence of a functioning implant prevented this loss. The sudanophilic zone of the fasciculata decreases markedly after hypophysectomy from 75% of the cortex width to 30% by 6 weeks after operation. The lipid-laden zone of the fasciculata was distinctly wider in animals with functioning implants than in hypophysectomized controls, though not as wide as in the normal animal.

260. THE NORMAL BLOOD PICTURE OF THE YOUNG ALLIGATOR (*ALLIGATOR MISSISSIPPIENS*) WITH OBSERVATIONS ON HEMATOLOGICAL EFFECT OF THIOUREA INJECTION. Philip H. Lieberman and Harry A. Charipper, Graduate School of Arts and Science, New York University, Washington Square.

The non-erythroid elements of alligator blood include thrombocytes, mononuclear leucocytes, basophils, lymphocytes and two varieties of acidophils. The nucleated red cells show a population of 602,500 per cubic millimeter with a variation of $\pm 38,500$ while the leucocyte count, including thrombocytes average $41,625 \pm 2850$. A modified supravital technique was used involving a diluting fluid of saline solutions of neutral red and crystal violet in equal parts in a Trenner red blood pipette. The probable homologous nature of the type II acidophile with its granular variation is suggested. Thiourea injections cause a lowering of the red and white cell count (RBC $441,500 \pm 30,650$; WBC $30,750 \pm 3000$) as compared to figures given above for untreated animals. The thyroid glands of these animals showed macroscopic enlargement following thiourea treatment with little change in relative amounts of colloids. It appears as though the histological changes are more hypertrophic than hyperplastic at least in so far as follicle size and number are concerned.

261. FURTHER STUDIES ON THE RELATION BETWEEN WAKEFULNESS AND THE GONADAL CYCLE IN BIRDS. Albert Wolfson, Northwestern University.

The results of previous experiments (Wolfson, '48) demonstrated that enforced wakefulness did not induce gonadal recrudescence in the slate-colored Junco (*Junco h. hyemalis*). The English Sparrow, however, did respond, but the results were inconclusive since no controls were used for this species.

Two more experiments have been performed, one designed to determine more accurately the response of the English Sparrow, the other to determine the role of wakefulness in the maintenance of the gonad after it was enlarged or had reached breeding condition.

In the experiment with the English Sparrows the controls responded as well as the experimentals. These results indicate that wakefulness itself is not a stimulus for gonadal recrudescence in this species. However, in the light of other recent findings, further experiments are needed to establish this conclusion.

The results of the other experiment showed that wakefulness did not maintain initially enlarged testes in the White-crowned Sparrow. In the English Sparrow, however, there was some evidence of maintenance of the breeding condition by means of wakefulness, when the breeding condition was induced experimentally in the winter by the means of light.

262. SERUM UPTAKE OF RADIO-CALCIUM IN GUINEA PIGS.¹ R. V. Talmage, J. W. Beck and E. T. Adams, The Rice Institute.

Considerable disagreement exists among investigators as to whether or not estrogen raises the serum calcium level in mammals. Preliminary experiments in this laboratory support the concept that estrogen does not effect the serum calcium level in guinea pigs, but that it does have a marked effect on the turnover rate of calcium in the blood. Female guinea pigs, castrated before reaching the weight of 350 gm and given at least a month's post-operative rest, were used. Estradiol was administered in 4 daily doses of 0.8 μ g and an open vagina was required for use on the 5th day. After 24 hours starvation all animals were administered, by stomach tube, as estimated 6 μ c of Ca^{45} in calcium lactate. Blood was removed by heart puncture at stated intervals later and serum values for total calcium and radio-calcium obtained. Ten controls gave an average serum calcium level of 11.05 mg percent ranging from 8.9 to 11.8. Serum calcium of 6 animals receiving estrogen averaged 10.7 mg percent ranging from 10.2 to 11.8. Radio-active measurements showed that in the controls, approximately 0.1% of the administered dose remained per milliliter of serum during the first 6 hours, dropping off gradually to a non-detectable amount at 24 hours. In the estrogenized animals there was an immediate high Ca^{45} value, reaching approximately 0.4% per milliliter of serum three hours after administration followed by a rapid fall; so that by 6 hours less than 0.1% remained.

¹ Aided by a grant from the U. S. Atomic Energy Commission.

263. OPPOSING ACTIONS OF PITRESSIN AND CORTICAL EXTRACT IN HYPOPHYSECTOMIZED RATS.¹ W. J. Eversole and C. M. Osborn, Syracuse University.

These experiments represent a further study of the apparent antagonistic action that exists between the antidiuretic posterior pituitary hormone and the diuretic adrenal cortical hormone(s).

Sixty adult male rats that had been hypophysectomized 18 to 73 days were divided into groups of 6 to 12. In each experiment the rats were fasted 12 hours then given water (5% body weight) by stomach tube twice, with an hour intervening. Cortical extract was administered as part of the water dose, whereas, Pitressin in saline was injected intraperitoneally. Following the second water dose, urine output was recorded for 4 hours at 30 minute intervals.

The majority of the animals received both cortical extract (4 cc) and Pitressin (40 mu). One group of controls was given cortical extract and saline and another group was given saline alone.

The most significant findings may be summarized as follows:

1. All rats receiving cortical extract excreted significantly more urine than saline controls despite Pitressin treatment.

2. Forty milli-units of Pitressin was most strongly autidiuretic in the cortical extract treated animals when given in divided doses of 30 milli-units within one hour preceding the first dose of water and 10 milli-units within one hour following the second hydration. Diuresis was reduced by about 50% in these experiments.

3. Pitressin antagonism lasted about two hours following injection.

4. Whether Pitressin and cortical extract have independent competitive effects upon the same target organ (kidney?) or act more indirectly in influencing water metabolism is to be studied further.

¹ This investigation was supported in part by a research grant from the National Heart Institute, U. S. Public Health Service.

264. TEMPERATURE STUDIES IN DWARF MICE.¹ D. H. Schonholz and C. M. Osborn, Syracuse University.

Studies of rectal temperatures were made on 76 genetically hypopituitary dwarf mice (Bar Harbor Strain) ranging in age from one day to 80 or more. A thermocouple in 1 mm glass tubing used with a potentiometer (Leeds and Northrup) proved satisfactory for measuring rectal temperatures. Control measurements were recorded on normal littermates.

In the young dwarfs, temperatures low at birth (32.9°C.) elevate most rapidly during the first 10 days of life; slowest from 10 to 25 days (while little or no weight increase occurs); and ascend slowly but steadily thereafter to the maximum (36.8°C.). The highest temperatures recorded from dwarfs averaged 1°C. below normal littermates. When body weights and rectal temperatures are plotted on one axis against age in days it is seen that the periods of most rapid weight increase coincide with the age when temperatures rise most rapidly toward the maximum for the adult. Maximum temperatures are reached at an earlier age (35 days) in the normal littermates than in dwarfs (50 days).

Thyrotrophic hormone (Armour,² lot No. TSH 2-D-3) administered subcutaneously in sufficient quantities to accelerate growth, produced also an elevation in rectal temperatures of about 1°C.

These observations are consistent with the work of Boettiger ('41) who found that dwarfs maintained at a high room temperature grew at the same rate as thyroxin treated animals.

¹ This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

² Courtesy of Dr. P. L. Munson, Research Section, Armour and Company.

265. THE EFFECTS OF THE THREE METHYL ETHER OF BIS-DEHYDRODISYNOLIC ACID (MDDA) ON THE REPRODUCTIVE SYSTEM OF *RANA PIPPIENS*. Alfred A. Renzi and Hiram J. Evans, Syracuse University.

In a study of the estrogen-like properties of MDDA its effect on both tadpoles and adults of *R. pipiens* was observed. The effect of the adult was compared with that of ethinyl estradiol.

Tadpoles were raised from the 18–20 mm stage to metamorphosis in the following aqueous solutions of MDDA: (a) 1 gamma of MDDA/liter of water, (b) 10 gamma/liter of water, (c) 100 gamma/liter of water and (d) 1000 gamma/liter of water. No significant alteration of the male-female sex ration was detected. Histological study of the gonads failed to reveal any tendency for inversion or reversal of sex. The Mullerian ducts were similar in both treated and control groups. It is concluded that under the conditions of these experiments, MDDA does not influence the development of the tadpole reproductive system.

Adult frogs were given 1000 gamma of MDDA by stomach tube every three days for a period of 26 days. Other adults received 100 gamma of estradiol by intraperitoneal injection every three days for a similar period. The controls were untreated. The reproductive system of the females treated with MDDA showed no noticeable stimulation, while the estradiol-treated group showed typical stimulation of the oviducts. In the adult males, both the MDDA- and estradiol-treated groups showed definite stimulation of the vestigial oviducts. MDDA produced slightly greater stimulation than did estradiol.

266. STUDIES ON THE EFFECTIVENESS OF DESOXYCORTICOSTERONE ACETATE (DCA) REPLACEMENT THERAPY IN ADRENALECTOMIZED RATS FED DIETS HIGH IN PROTEIN BUT FREE OF CARBOHYDRATES.¹ Elizabeth F. Place and W. J. Eversole, Syracuse University.

Both mature and immature rats were fed one of three synthetic diets. Diet 1 contained 84% casein; diet 2, 84% vitamin-test casein; and diet 3, 84% lactalbumin. Most of the animals were adapted to a purified diet previous to adrenalectomy but others were given the diets immediately after the operation. Animals were placed in individual cages, except in a few instances where two animals were housed in one cage. Records on body weight and food consumption were taken daily. Blood samples were drawn at intervals and the following determinations were made: hematocrit, hemoglobin, serum protein, non-protein nitrogen and sugar.

Adrenalectomized rats treated with DCA (0.2–0.5 mg/day) and fed high protein diets survived a 20-day experimental period and gained weight; animals fed lactalbumin showed the poorest growth response. In non-fasted animals the blood values were mainly within normal range. Under fasting conditions, the blood sugar levels of adrenalectomized treated rats on any type of diet were markedly below those of intact rats on similar diets.

These results vary somewhat from earlier reports (Eversole, '45; Segaloff, '46) in which adrenalectomized DCA-treated animals from other strains, under different

laboratory conditions, did not survive when fed certain high-protein diets. The basis of the discrepancy is not understood.

¹ Aided by a grant from the Penrose Fund of the American Philosophical Society.

267. PROPERTIES OF THE ANTIDIURETIC SUBSTANCE IN THE SERUM OF NORMAL RATS.¹ James H. Birnie and W. R. Boss, Syracuse University. (Introduced by Robert Gaunt.)

Some properties of the antidiuretic substance (ADS) present in the blood of normal rats have been described by the authors and collaborators. An attempt was made to determine additional properties such as the effect of serum ADS on renal function.

The injection of hydrated test rats with 1 cc of fresh serum from normal rats caused a slight increase in glomerular filtration within 30 minutes after administration as indicated by creatinine clearance (injected = 1.231 cc/100gm/minute; control = 0.956). During the same period there was a marked decrease in the rate of urine flow (injected = 0.026 cc/100 gm/minute; control = 0.062). These results indicate that the action of serum ADS is to increase the tubular reabsorption of water rather than to decrease glomerular filtration. In this respect, as in others previously reported, serum ADS resembles posterior pituitary extract.

When serum obtained from normal animals was allowed to stand for 30 minutes at 23°C. before being injected into hydrated test rats it caused no alteration in glomerular filtration and had lost considerable portion (75%) of its antidiuretic activity. If posterior pituitary extract (Pitressin) is added to serum and allowed to stand in a similar manner its antidiuretic activity is lost.

When fresh serum was mixed with 0.01 M thioglycolic acid which had been neutralized to pH 7.5 and allowed to stand for 5 minutes at 23°C. the serum lost its antidiuretic activity. Posterior pituitary extract (Pitressin) treated in a similar manner with thioglycolic acid lost its antidiuretic activity. While these results are consistent with the hypothesis that serum ADS is posterior pituitary hormone, further evidence on the point is being sought.

¹ Supported by a grant from the National Heart Institute, U. S. Public Health Service.

268. INTRAOCULAR HOMOPLASTIC TRANSPLANTATION OF THE ANTERIOR LOBE OF THE FROG PITUITARY. Robert F. Kallman, Albert S. Gordon and Harry A. Charipper, Department of Biology, Washington Square College of Arts and Science, New York University.

Pituitary glands of adult winter frogs (*Rana pipiens*) were transplanted homoplastically to the anterior eye chambers of host animals. Donors were decapitated, the hypophyses exposed, bisected *in situ*, and a half gland transferred immediately through a previously made corneal incision into the eye of an otherwise intact host of the same sex.

Most of the implants were well vascularized and displayed iridial pigment migration. Representative implants revealed several common features. Persistence of typical basophilic and acidophilic elements in nesting arrangement was observed (in one case for more than 60 days), although some degeneration was noted in all implants. There also occurred a general loss of compactness, a development of a fibrous intercellular reticulum, an increase in macrophagic activity, and the formation of a connective tissue stroma. Complete resorption of living implants was rarely observed. In some of the long-term implants, well-defined chromophilic cells were detected despite the absence of a new vascular supply.

It is concluded that histological maintenance of pituitary tissue implanted into the eye chamber of the frog can occur even in the presence of the animal's own pituitary.

Genetics

269. WEIGHT VARIATION BETWEEN PIEBALD AND NORMALLY COLORED HAMSTERS. Charles L. Foote and Harrison E. Bullock, Southern Illinois University.

In a preliminary report (Foote, '49) a coat color change in the golden hamster has been described. The usually brown colored hairs on the underside of the throat, on the face, and areas on the back are replaced by white hairs. This condition has been termed piebald and animals graded from 0 (normal) to 10 (no brown hair on underside with a great amount of white on face and back). The piebald condition is thought to be a mutation since these animals differ from normals in other respects than in coat color. One apparent difference is in weight. A group of 51 animals, 27 of grades 3 to 10, and 24 of grades 0 to 2, was chosen for weight studies. All animals were kept under identical conditions.

Animals of grades 3 to 10 had an average weight of 24 gm at 28 days of age as compared to a average of 35 gm for the 0 to 2 group. This weight difference was maintained throughout the period during which the animals were observed. At age 75 days the weight difference between the two groups was 14 gm. Twelve animals (8 of grades 3 to 10, and 4 of grades 0 to 2) of the original 51 animals showed an average weight difference of 18.4 gm when 320 days old.

Some individual piebald animals do attain a weight equal to the average of the 0 to 2 group, but litter-mates of such animals always weigh more than the group average.

No explanation for the weight difference between piebald and normally colored hamsters has as yet been determined, although weight variations in different genetic strains in other species have been reported.

270. PARALLEL DISTRIBUTION OF GENE FREQUENCIES IN 7 SPECIES OF COLIAS BUTTERFLIES. William Hovanitz, University of San Francisco.

Populations of *Colias* butterflies are heterogeneous for a pair of alleles which have color, and physiologically-adaptive, phenotypical effects upon the adults. In each of 7 species, the frequency of the allele for white wing color is more

common than that for yellow or orange in comparable geographical and ecological locations. A reasonable amount of hybridization occurs between the species which permits transfer of genes between them. However, the parallel distribution of the genes seems to be due more likely to selection of the genotype by the direct action of the physical factors of the environment. These factors seem to have a similar influence in each of the 7 species.

General Morphology

271. THE OONOPIDAE OF PANAMA. A. M. Chickering, Albion College.

The Cambridges (1889-1905) had no Oonopidae from Panama. Petrunkevitch ('25) described *Oonops reticulatus* from Panama City. Banks ('29) reported *Dysderina plena* O.P.-Cambridge from the Canal Zone. Gertsch ('41) described *Scaphiella barroana* and *S. williamsi* together with *Oonopinus centralis* from the Canal Zone Biological Area. He also reported the presence of *Dysderina plena* from the same locality. From my personal collections together with valuable additions furnished by Dr. James Zetek the following new species have been determined and are carefully described in this paper: *Dysderina dura* sp. nov.; *D. recondita* sp. nov.; *D. seclusa* sp. nov.; *D. silvatica* sp. nov.; *Oonopinus modestus* sp. nov.; *O. pallidulus* sp. nov.; *Oonopoides bryantae* sp. nov.; *Oonops donaldi* sp. nov.; *O. zeteki* sp. nov.; *Opopaea bequaerti* sp. nov.; *O. recondita* sp. nov.; *Scaphiella gertschi* sp. nov.; *Stenoonops petrunkevitchi* sp. nov. The author deems it unlikely that *Dysderina plena* O.P.-Cambridge occurs in Panama.

272. GROWTH OF THE KIDNEYS IN THE FETAL DOG. Homer B. Latimer, University of Kansas.

Both kidneys were removed, free of fat and attached vessels, from 156 fetal and 24 newborn dogs. Four specimens were too small for positive sex identification but the remainder were equally divided as to sex. The kidneys were weighed in glass stoppered weighing bottles, to a milligram for the larger specimens and to a tenth of a milligram for the smaller fetuses.

The weights of the two kidneys were plotted on body weight and on body length. When plotted on body weight the resulting curve is composed of two straight lines, the second having a slightly greater pitch. When the body length is used as abscissae the curve is concave superiorly, rising very slowly at first and more rapidly in the largest fetuses. Empirical formulae will be presented for these curves.

The kidney weights as percentages of the body weight were plotted on both body weight and on body length. The percentage weights increase in the first part of the period and then decrease in rate of change. Plotted on body weight, the resulting curve is very irregular at first and then smooths out. Plotted on body length the resulting curve is nearly a straight line at first and again flattens out toward the end. There are no apparent sex differences, and the weights of the newborn kidneys fit in well with the weights of the fetal kidneys.

273. VARIATIONS IN THE NUMBER PER LITTER AND IN THE INDIVIDUAL WEIGHTS IN EACH LITTER, IN A SERIES OF DOG FETUSES. Homer B. Latimer, University of Kansas.

A series of 156 fetal and 24 newborn puppies came from a total of 37 litters. There were 30 litters of fetal dogs, with from one to 9 per litter and averaging 5.2 fetuses per litter. The 7 litters of newborns ranged from one to 6, and with an average of 3.4 puppies per litter. Three litters of fetuses had 9, and three had 8 per litter. Two litters had but one specimen.

The ratio of the largest specimen in each litter to the smallest ranged from 1.078 to 2.605 with an average of 1.31.

The average percentage deviation of each specimen from the average of the litter ranged from 1.71 to 22.77%, with an average of 7.63. The two litters of one each were not included.

The ratios and the percentage deviations were plotted on the number of specimens per litter and on the average body weight of the litters and in all 4 cases no discernable trend of these values was found. There was just a general scatter.

The number of litters is not adequate for final conclusions, but these data seem to indicate that neither the number in the litter nor the average body weight of the litter determine the individual variations in number and in size within a litter. These were mongrels and of unknown parentage, taken from a series of dogs used in physiology experiments, and the variability in the litters may be due to the inheritance of size factors from the parents.

274. SEXUAL DIMORPHISM IN THE ANAL FIN AND ANAL FIN SUSPENSORIUM OF ANABLEPS ANABLEPS. C. L. Turner, Northwestern University.

Sexual dimorphism in the anal fin and fin suspensorium of *Anableps anableps* is extreme. The 11 rayed unspecialized fin of the adult female is much like that of the embryo but the 12 rayed fin of the mature male is displaced laterally and is modified into a highly specialized gonopodium. Most of the rays are curved, twisted and locally thickened and the entire complex is folded about an enclosed tube. The gonopodial suspensorium also contains specialized structures. The anterior interhemal rays are elongated and the first 4 are partially enclosed by wing shaped extensions of the first ray. The hemal spines of the suspensorium differ from those of the female in inequality of length and possession of lateral extensions and median spurs.

275. THE MORPHOLOGY AND HISTOLOGY OF THE PANCREAS OF THE PLATYFISH, *PLATYPOECILUS MACULATUS*. Gladys Reiter and Ross F. Nigrelli. Graduate School of Arts and Sciences, Washington Square College, New York University and New York Zoological Society.

The exocrine and endocrine parts of the pancreas of the teleost, *Platyopocilus maculatus*, are separate structures. Histologically and cytologically the pancreatic cells are typical of other vertebrates. However, they are scattered more or less diffusely along the mesentery, sometimes closely adhered to the intestine and

liver and completely surrounding the gonads. They line the larger blood vessels which pass into the liver and also follow the bile duct to the duodenum. The endocrine part is a single encapsulated body situated in the region between the liver and the duodenum at the level of the bile duct. The gland is highly vascularized and morphologically consists of one type of cells arranged in cords. When stained with Gomori's, the cells grade in staining reaction from a basophilic to an eosinophilic condition, with the former predominating. Evidence of secretion in the embryonic exocrine pancreas was noted.

General Physiology

276. THE EFFECT OF SMALL DOSES OF GAMMA RADIATION ON THE RESPIRATION AND DEVELOPMENT OF THE PREDIAPAUSE EGGS OF GRASSHOPPERS. Theodore N. Tahmisian and Jeanne D. Gasvoda, Argonne National Laboratory.

When prediapause eggs of grasshopper were irradiated with small doses of γ radiation from radium (4.5 to 52.5 r) there was some inhibition of respiration when measurements were made soon after irradiation. This inhibition was reversible. The amount of radiation used in these experiments represents the lowest dose possible to produce alterations in the metabolism of grasshopper eggs.

Soon after irradiation 6-day embryos showed pyknosis of the nuclei and inhibition of mitosis. Mitosis was again present 24 hours later.

Two hours after irradiation 7-day and 9-day embryos showed pyknosis as well as cells in mitosis, an indication of less alteration than that produced in 6-day embryos. This pyknosis was still visible 24 hours after irradiation.

Few pyknotic nuclei were seen in 10-day and 11-day embryos.

Embryos irradiated with 4.5 to 52.5 r of γ radiations entered the diapause phase as the normal cells, an indication that morphological and functional alterations produced by small amounts of radiations are reversible.

277. FIXATION OF $C^{14}O_2$ IN GRASSHOPPER EGGS. Theodore N. Tahmisian and Donald E. Buchanan, Argonne National Laboratory.

The determination of the ratio $\frac{\text{air carbon}}{\text{food carbon}}$ that exists in organisms would be of considerable value in fixing $C^{14}O_2$ tolerance levels. To obtain this ratio for steady states by means of tracer experiments it is necessary to place organisms in a constant and labeled atmosphere until the measured ratio becomes constant.

This was done in a preliminary fashion by incubating eggs of *Melanoplus differentialis* in a sealed system of large volume containing $C^{14}O_2$. The results seem to indicate that the ultimate ratio attained with the tracer is not widely different for tissue in a steady state as compared with growing tissue, but that the growing tissue approaches the ratio at a much greater rate.

278. THE EFFECT OF β IRRADIATION ON THE RESPIRATION AND MORPHOLOGY OF MELANOPLUS DIFFERENTIALIS EMBRYOS. Theodore N. Tahmisian and Jeanne Gasvoda, Argonne National Laboratory.

Low dosages of β radiation produced extensive damage in the embryonic cells of grasshoppers. The respiration of grasshopper embryos was increased during irradiation; immediately after irradiation there was inhibition. By the use of Flemming's triple stain it was shown that β irradiation caused pyknotic chromatin to remain as if it were in the metaphase condition. There is evidence that injury sustained by the chromosomes may be direct and indirect. β irradiation caused the appearance of some osmophilic material associated with the chromosomes. The nature of this material is as yet unknown.

279. THE EFFECT OF PH ON THE SODIUM AZIDE AND SODIUM CYANIDE INHIBITION OF RESPIRATION IN ORYZIAS LATIPES. Margwyn Harris and S. Milton Nabrit, Atlanta University.

Four day old embryos (25°C.) were subjected to an HCl adjusted range of pH values in $\frac{M}{100}$ solution of sodium cyanide and sodium azide. Heart beat frequency was determined after 5 minutes.

In cyanide maximum inhibition was at pH 5.3 and the minimum at pH 6.65. The inhibition was not graded from low to high pH range.

In azide a graded decline in inhibition as indicated by heart frequency occurred as the pH value increased from a pH 5.03 to a pH of 11.4.

Greater inhibition was obtained from cyanide than from azide.

Changes in hydrogen ions from the HCl normally influences respiration, but the pK's of hydrozoic and hydrocyanic acids are also influenced by H ion concentration. At 25°C. hydrocyanic acid has a lower dissociation constant than hydrozoic and most of the inhibition appears to be due to the free acid which probably accounts for greater inhibition of cyanide at low pH values.

280. FATTY ACID AND PROTEIN SYNTHESIS IN CHILOMONAS PARAMECIUM. B. J. Sullivan, Boston College. (Introduced by Sr. Elizabeth Seton MacDonald.)

The growth of *Chilomonas paramecium* in sterile acetate-ammonium medium has been the subject of several recent papers. The present investigation deals with an estimation of the amount of fatty acid and protein synthesized, and the correlation of the food synthesis curve with the growth curve of the organism.

A modified Bloor method was used to estimate the amount of fatty acid. Values obtained indicate that there is $10^{-5} \times 1.271$ mg of intracellular fatty acid per 1000 chilomonads. This is equivalent to 5.02% of the wet weight and 20.35% of the dry weight of the organism. The total fatty acid produced by the organism at first increases in direct proportion to the increase in population. This curve levels off when the population reaches 20,000 organisms per cubic centimeter and declines sharply when the population reaches 35,000 organisms per cubic centimeter. The decline continues as the population increases to a maximum density of 40,000 organisms per cubic centimeter.

The amount of total protein was estimated by the method of Andersch and Gibson. A mean of $10^{-3} \times 2.617$ mg per 1000 chilomonads was obtained. This represents 11.12% of the wet weight and 44.98% of the dry weight of the organism.

An initial peak of protein synthesis is reached in populations of 20,000 organisms per cubic centimeter. This is followed by a decline in cultures having populations of 25,000 and 30,000 organisms per cubic centimeter. As the population increases above this point protein synthesis increases until a second, and higher, peak is obtained in the maximum population level of 40,000 organisms per cubic centimeter.

281. MECHANISM OF ACTION OF ANTIHISTAMINIC DRUGS.¹ T. C. Barnes, Hahnemann Medical College and Hospital of Philadelphia.

Previous reports (Barnes, Anat. Rec., 101: 740, 1948; Federation Proceed. 7, 204, 1948) described the adrenergic property of antihistaminics. These drugs dissolve in triglyceride oils (triacetin, tributyrin and tricaproin) in which adrenergic but not cholinergic drugs are soluble. Penetration and ionization in the oil produce a negative electrical phase-boundary potential which may exert the pharmacodynamic action. Animal experimentation has shown the power of these drugs to antagonize histamine but some of the effects may be due to the activation of adrenergic nerve endings (producing relaxation of smooth muscle, etc.). The shape of the curve relating pharmacological effect to concentration is claimed to resemble that of adsorption suggesting substitution of antihistaminic for histamine by adsorption on a receptor. This is no proof for the adsorption theory as the following data show. Equal parts saline and 0.1% sodium oleate (volume 200 cc) established a positive potential of 20 millivolts at nitrobenzene interface. One milligram of antistine (Ciba) added to the 200 cc $\times$ 62 produced 62 millivolts negativity. The addition of 4 more increments of 5 mg each of antistine produced the following changes in the total potential -94, 112, 122 and 137 millivolts. Thus the exponential dosage curve found *in vivo* might be produced by a phase-boundary potential.

¹ This research was supported by a grant from Ciba Pharmaceutical Products, Inc.

282. RESPIRATION AND WEIGHT CHANGES IN PHORMIA LARVAE AFTER EXPOSURE TO DDT. John B. Buck, Margaret L. Keister and Irene Posner, National Institutes of Health, Bethesda, Maryland.

Mature larvae of the bluebottle fly, *Phormia regina*, were exposed to DDT (as crystals, water suspension, or solution in kerosene or olive oil) by surface contact, injection into tracheae, injection into blood, or by feeding. The body cuticle was found to be completely impervious to DDT in any form, but larvae could be fatally poisoned by injection into either tracheae or blood. DDT was practically non-toxic by mouth.

Poisoned larvae showed a two- to three-fold increase in oxygen uptake, and an increase in R.Q. from 0.74 to 0.84. As in mature flies, these changes are probably attributable to the induced hyperactivity. Both oxygen uptake and weight loss were significantly higher in low than in high humidity. Although poisoned larvae gained weight in water, this is attributed to leakage of water through the spiracles into the body, rather than to increased cuticular permeability. The evidence suggests that DDT impairs the hydrophobic properties of the spiracular wax.

283. CULTURE OF MANTLE TISSUE OF MARINE MOLLUSCS. Gerrit Bevelander and Josephine Martin, New York University.

Culture of the mantle of several species of marine molluscs was undertaken in connection with studies dealing with the mechanism of calcification. After experimentation with culture conditions such as media, temperature, etc., tissues were eventually maintained in viable states exhibiting muscular and ciliary activity in mollusc blood plasma at temperatures approximating that of the sea water from which they were removed.

Within the first 24 hours, cultures containing excised strips of the mantle of *Pintata radiata*, for example, show the following characteristics: (1) extrusion and clumping of epithelial, pigment, and blood cells; (2) elaboration of fibrous material (conchin); (3) secretion of mucus.

Cultures maintained for longer periods, 4-6 days, frequently exhibit the presence of crystal aggregates typical of those found in normal or regenerating shell. In the majority of these preparations the crystal formation occurs some distance away from the mantle edge, and usually in an exudate consisting of mucus.

284. DISTRIBUTION OF FREE AMINO ACIDS IN NERVE TISSUE. Eugene Roberts and Pinckney J. Harman, Washington University School of Medicine, New York University College of Medicine, and Roscoe B. Jackson Memorial Laboratory.

A survey was made by two-dimensional paper chromatography of the free amino acids found in alcoholic extracts of whole brains from mice of three inbred strains and of the gray matter, diencephalon, spinal cord, and sciatic nerve in a normal and ataxic rabbit.

Glutamic acid and taurine were present in the greatest quantities in adult mouse brain. Relatively large amounts of aspartic acid, glutamine, and histidine were also found. Somewhat smaller concentrations of glycine, serine, and alanine were noted, and very small quantities of cystine, valine, and the leucines were detected. The high content of free histidine in brain is of special interest, since only traces of this amino acid have been found in 20 other tissues examined. The distribution of amino acids in the brains of 15-day fetuses resembled that obtained for placenta and fetal liver, but was different from that found in the brains of weanling or adult mice. An intermediate pattern was found in the day-old mouse.

The patterns found in the gray matter and spinal cord of the normal rabbit were very similar to each other and resembled those found in mouse brains, with the exception that taurine was present only in traces in the rabbit tissues. There

were much smaller concentrations of free amino acids in the diencephalon and in sciatic nerve. Cerebral cortex and spinal cord, which have relatively large amounts of the free amino acids listed above, showed marked reductions in these substances in the ataxic rabbit.

285. RESPONSES OF THE RED CHROMATOPHORES OF THE FIDDLER CRAB. Frank A. Brown, Jr., Muriel I. Sandeen, and H. Marguerite Webb; Cresap Biological Laboratory, Northwestern University, and Marine Biological Laboratory, Woods Hole, Mass.

Unlike for the black and the white chromatophores concerning which much is known, the behavior of the red chromatophores of *Uca pugilator* has not been studied previously in any detail. The condition of the red chromatophores of normal animals upon a black background and upon a white background, as well as the effect of injection of extracts of various parts of the nervous system and of the sinus glands, was studied. The red chromatophores became broadly, though usually not completely, dispersed in normal animals upon a black background. Upon a white background the chromatophores became completely concentrated. Removal of the eyestalks results in permanent concentration of the red pigment. Extracts of sinus glands, optic ganglia, supraesophageal ganglia, or thoracic cord, when injected into eyestalkless animals, brought about, after a delay of approximately 30 minutes, rapid dispersal of the red pigment. Extracts of the circumesophageal connectives showed little or no effect. When injections of similar extracts were made into animals which had previously been adapted on a black background, it was found that an initial extensive concentration occurred in all cases. This concentration was followed by rapid dispersal to complete dispersion, and then a return to the original condition two to three hours following injection, for all extracts except connectives. In the last the dispersing activity was absent.

These experiments provide strong support for an hypothesis that all these organs except the connectives contain both concentrating and dispersing principles for red pigment; the connectives contain significant quantities of the former only.

286. A PERSISTENT RHYTHMICITY IN *UCA* RED CHROMATOPHORES. Frank A. Brown, Jr., J. Bruce Guyselman, and Muriel I. Sandeen, Cresap Biological Laboratory, Northwestern University, and Marine Biological Laboratory, Woods Hole, Mass.

The presence of a diurnal rhythm of activity of the black and white chromatophores of *Uca pugilator* has been reported and characteristics of the rhythm rather extensively studied. However, up to the present time, nothing has been reported regarding the occurrence of a similar rhythmicity for the red chromatophores. With the intention of determining whether such a rhythm existed, normal specimens of *Uca pugilator* were maintained in constant darkness and the state of the red pigment observed at intervals throughout the day and night. It was found that the red pigment was completely concentrated at night and

more or less broadly dispersed in the daytime. This rhythm was observed to persist for at least 5 days. The states of dispersion of the pigment in the day and night phases of this rhythm are qualitatively the same as those previously reported for the black and white chromatophores.

The presence of a diurnal rhythm of activity in constant darkness is of particular interest in this case in view of the fact that, unlike for black and in good measure for the white, the degree of dispersion of the red pigment under conditions of normal daytime illumination is almost entirely dependent upon a response to background.

287. BLACK CHROMATOPHORES OF *UCA* AS INDEPENDENT EFFECTORS. Frank A. Brown, Jr., J. Bruce Guyselman, and Muriel I. Sandeen, Cresap Biological Laboratory, Northwestern University, and Marine Biological Laboratory, Woods Hole, Mass.

Recent experiments (Brown and Sandeen, *Physiol. Zool.*, 4: 361, 1948) have shown that total illumination is one of the environmental factors which influence the state of dispersion of the pigment within the black chromatophores of normal *Uca pugilator*. In order to resolve the question of whether this phase of the response is due to the influence of illumination upon the chromatophores themselves, or is due to an indirect action of the light which is mediated by an integrating mechanism within the animal, a restricted region of the body was shielded. Such experiments involved the use of a short section of black rubber tubing as a shield over the proximal segments of a leg. Eyestalkless specimens have their black pigment completely concentrated at illuminations between zero and about 40 f.c., and when illumination is increased to 2000 or 3000 f.c. have their black pigment about half dispersed. Shielded portions of legs of such eyestalkless animals fail to exhibit the typical pigment dispersal at the higher illuminations. Thereby it is demonstrated conclusively that there is an independent-effector activity of these chromatophores in response to light, and that this activity is a significant factor in the normal responses of the chromatophore system.

288. VAGAL TONICITY IN THE TURTLE. Jacob E. Wiebers, Betty Blue and William A. Hiestand, Purdue University.

Twelve turtles (9 *Pseudemys scripta troostii*, two *Chrysemys picta marginata*, and one *Emys blandingii*) were used, none of which was anaesthetized or any portion of the brain destroyed throughout the experiments. The vagi were exteriorized from the base of the neck by removing the overlying skin and fascia. The plastrons were removed posteriad the pectoral shields by cutting with a cranial forceps. The heart beat was recorded on a kymograph by attaching a heart lever to the frenum of the heart.

No tachycardia occurred after single or double vagotomy. The left vagus was severed first, since it is the less active when stimulated. In two out of three cases heart blocks developed after sectioning of the left vagus. In 4 of of 12

turtles heart blocks were present before the onset of the experiment. Vagal stimulation was found to abolish the heart block, section of the left vagus would reduce the number of heart blocks and in all cases double vagotomy resulted in complete abolishment.

No apparent cardiac acceleration was observed in turtles after atropine was applied peripherally to the vagi. The medullary centers were stimulated by atropine, causing a primary bradycardia. There was no indication of any tachycardia with the vagi paralyzed. Cocainization of the vagal trunks had no effect on cardiac rate. Thus evidence is lacking of any vagal tonicity in the turtle.

289. THE EFFECT OF INANITION AND FOOD AND WATER DEPRIVATION ON THE SURVIVAL TIME AT LOW TEMPERATURES IN THE ALBINO MOUSE. Nathan Primack and William A. Hiestand, Purdue University.

Food is of prime importance for the survival of the animal at low temperatures, water apparently being of lesser importance. Thirty albino mice of the Hygienic strain were placed in a cold room where the temperature was kept at 8–10°C. Humidity remained constant at 88–94% as determined by the sling psychrometer. Mice were divided into two equal groups, those receiving water and no food and those receiving neither food nor water. For a period of 7 days both groups were maintained in the cold room on food (Purina Laboratory Chow) and water ad libitum. They were then starved for 8 hours, fed for two, and then weighed and placed in individual cages. The total weights of the two groups varied only 0.4 g. The first group of mice was fed 0.5 ml of distilled water intragastrically at 12 hour intervals beginning with the first 12 hours following the two hour feeding period. Force feeding was continued until death and was necessary because of the failure of the mice to drink in the absence of food at low temperatures. It was found that the group receiving water survived an average 42.4 hours while those of the other group survived 36.9 hours. The difference in survival was not significantly large to assume that water had prolonged survival. Fisher's *t* being 0.49 with an *N* of 8 gave a probability of 65%.

290. THE EFFECTS OF VERATRUM VIRIDE ON THE BLOOD SUGAR LEVEL OF THE ALBINO RAT. David E. Mann, Jr., Arthur G. Zupko, Phillip V. Hammond and William T. Rockhold, Purdue University. (Introduced by William A. Hiestand.)

Veratrum viride acts as a cardiac depressant by stimulating the vagal centers of the medulla resulting in decreased pulse rate and blood pressure. Once used clinically to relieve hypertension, it has now been largely supplanted by drugs of lesser toxicity. Despite this fact, the pharmacology of V.v. is still being investigated.

The purpose of this experiment was to observe the effects, if any, of this drug on the blood sugar level of the albino rat. Twenty mature rats of both sexes were fasted for 12 hours prior to the withdrawal of blood from the tails. The 10 animals in Group 1, ranging in weight from 169 to 340 gm, were in-

jected intraperitoneally (immediately after the initial withdrawal) with a 0.1 mg/kg dose of a freshly prepared aqueous solution of V.v. (1 mg/cc). Twenty minutes after each injection, a second blood sample was obtained by decapitation. The 10 animals in Group 2 (247 to 390 gm) underwent the same procedure with the exception that they were injected ip. with an aqueous solution of V.v. that had been refrigerated for three days. Blood sugar determinations were made by the Folin-Wu micro method.

Freshly prepared aqueous solutions of V.v. caused hypoglycemia within 20 minutes in mature rats which had fasted for 12 hours. The average blood sugar decrease was 30.6 mg%. Aqueous solutions of V.v. which had been refrigerated for three days caused hyperglycemia with an average blood sugar increase of 41.8 mg%.

291. EFFECT OF ANESTHETICS ON THE WATER-HOLDING PROPERTIES OF FROG MUSCLE. Walter Grundhauser, St. Louis University. (Introduced by Basile Luyet.)

In a series of studies of the factors which may permit protoplasm to support advanced dehydration, anesthetics (which are known to modify permeability) were investigated as possibly modifying the water-holding properties of cells. Sartorius muscles were immersed in frog's Ringer containing 0.1% chloroform and subsequently placed in dessicators at room temperature. They lost an average of 26.1% of their weight in one-half hour, while their non-anesthetized controls lost 31.2%. Similar experiments with ether yielded comparable results. In a second series of experiments gastrocnemius muscles were dehydrated in the presence of the anesthetic at constant temperature, humidity, and pressure. They were suspended in closed jars over saturated CaCl_2 solution in an atmosphere of ether of known concentration, and the jars were immersed in a constant temperature bath at 30°C. The pressure in the control jars was made equal to the pressure in the experimental jars by the addition of air. In all cases, the muscles exposed to the ether vapors showed a smaller loss of weight (an average loss of 24.4% of their weight as compared to 35.1% in the controls). However, when the concentrations of the anesthetic were so high that the muscles would not recover, a fluid which gave the protein test was exuded and any determination of the decrease in weight by evaporation lost its significance. Thus, in general, anesthetics in weak doses seem to increase the water-holding properties of muscle.

292. ON SOME PROBLEMS CONCERNING THE LIFE CYCLE OF MYCETOZOA. P. H. Gehenio and B. J. Luyet, Biodynamica Research Laboratory and St Louis University.

In an attempt to elucidate some complex and confusing problems of the life cycle of Mycetozoa about which there are many contradictory statements in the literature, in particular, whether the spores are already sex-segregated when they are formed and whether the swarmers arising from the spores or the amoeblae resulting from successive divisions are gametes, we undertook a series of observations consisting in following the development of the organism from sin-

gle spores and from single amoebulae from any of the successive cell generations obtained from a single spore. Working with *Physarella oblonga* we obtained, so far, the complete cycle, including sporulation, from single spores, from single swimmers derived from single spores and from single amoebulae of the first 5 cell generations after spore germination. Isolated cells from all generations up to the 12th have developed into plasmodia which are growing normally. There has been no indication that the cells of these later generations form plasmodia before having divided again 10 to 12 times, which is about the number of divisions that the amoebula born from the original spore will undergo before forming plasmodia in undisturbed cultures. Isolated cells of the next generations up to the 25th are in the process of cell division preceding plasmodium formation and there is no evidence that the rate of division has decreased in them. Our observations thus indicate that *Physarella* forms plasmodia without gametic fusion in the course of the first 12 divisions after spore germination.

293. A STUDY OF THE GLUCOSE CONTENT OF THE BLOOD OF POMARINE JAEGERES FROM THE POINT BARROW REGION OF ALASKA. X. J. Musacchia, St. Louis University, Bernard J. Sullivan, Boston College and Louis A. Susca, The New York Medical College, Flower and Fifth Avenue Hospitals. (Introduced by Secretary.)

Wilber and Musacchia ('49) reported results of lipid metabolism in various Arctic Alaskan birds. In an effort to complete studies of various organs and tissues of Arctic birds, glucose analyses of the blood of the pomarine jaeger, *Stercorarius pomarinus*, were made during the summer of 1949. The investigation was made possible by the Arctic Institute of North America and the Arctic Research Laboratory, Office of Naval Research, Point Barrow, Alaska. Jaegers were obtained from the tundra in the Point Barrow area. Blood for analyses was taken from the ventricle of the birds immediately after the birds were killed with a shotgun. Estimations for glucose in the blood, using the Somogyi filtrate, were made on each of 14 animals. All results are expressed in mg per 100 cc of blood. The lowest value obtained was 130; the highest, 370. A mean (for 14 specimens) of 223.7 was obtained. Considerable variation occurs in the values obtained for the various birds. Perhaps, the age and sex of the birds, and the manner in which they were obtained, may be an explanation for the variation. Further studies on the tissues and organs of the birds are in progress.

294. A COMPARATIVE STUDY OF CHOLESTEROL IN SOME INVERTEBRATES. Louis A. Susca, The New York Medical College, Flower and Fifth Avenue Hospitals and Charles G. Wilber, St. Louis University. (Introduced by Secretary.)

In the past, investigators have confined their investigations on cholesterol changes chiefly to the homoiothermal animals and to different protozoa. Very little has been done on the problem in the invertebrates. A study of the cholesterol changes was made on three species of invertebrates: the common housefly, *Musca domestica*; the earthworm, *Lumbricus terrestris*; and the turtle leech, *Glossophonia* sp. Specimens were prepared for analysis by grinding

weighed amounts with sand in a mortar (Bloor, '29). The amount of cholesterol was estimated colorimetrically using the acetic anhydride-sulfuric acid reagent (Bloor, '28). Mean values (of 10 specimens from each species studied) for cholesterol, in milligram per cent of wet weight, are as follows: *Musca domestica*: 0.15; *Lumbricus terrestris*: 0.40; *Glossophonia* sp.: 0.72. The results indicate that there is a wide variation in the amount of cholesterol among different invertebrate species. Some of the results obtained in this investigation are interesting when compared to that obtained for cholesterol in the annelid *Chaetopterus variopedatus*, by Wilber and Bayors ('47). A mean value (for 10 worms) of 0.16 mg per cent of wet weight is given by Wilber and Bayors. Thus, the amount of cholesterol in *Chaetopterus variopedatus*, an annelid, is similar to the amount of cholesterol found in *Musca domestica*, an insect.

These results may indicate that cholesterol is a normal constituent of invertebrate tissue, as it is of vertebrate tissue. Further studies on the lipids in the invertebrates used in this investigation may prove interesting.

295. SUGAR IN THE BODY FLUID AND TISSUES OF A SEA URCHIN. Schuyler V. Hiltz and Arthur C. Giese, Department of Biological Sciences, Stanford University.

Strongylocentrotus purpuratus freshly collected in the vicinity of the Hopkins Marine Station, Pacific Grove, California and kept without food digests the food in its gut very slowly, continuing to defecate for over two weeks. The gut contains only traces of glucose at any one time. The body fluid of freshly collected urchins showed no glucose when tests were made with Somogyi micro method which would detect anything over 2 mg per 100 cc. Raising the temperature of the urchins to 18, 25, 28 or 32°C. did not increase the glucose content; at the two higher temperatures the sea urchins promptly died.

The saccharide content of tissues of the urchin determined as glucose after acid hydrolysis is highest in the gonads and the intestine (washed free of contents) being about 1.2-1.5%; the animal as a whole contains much less.

When a sample of 20 mg glucose per 100 gm of animal was injected into the body fluid, the tissues took up the glucose fairly rapidly, in one case 96% being removed in an hour; on the average 56% was removed in an hour. The hypothesis is put forth that the tissues of the animal live in a constant state of glucose starvation, the small traces appearing in the body fluid as a result of digestion being removed rapidly by the tissues.

296. ULTRAVIOLET RESISTENT BACTERIA IN ALBUMIN SOLUTIONS. Arthur C. Giese, Department of Biological Sciences, Stanford University.

Contaminations were observed in 1% bovine serum albumin solutions after prolonged irradiation with a Sterilamp, in the course of experiments on the effects of radiations from this source on protein decomposition. The solutions, made up sterilely with the exception of the albumin (amorphous, Armour and Co.), were continuously stirred with a magnetic flea during irradiation. The intensity

of the radiations was on the average over 100 ergs/mm²/sec. and exposures in several cases were over a week. Samples were taken from the central fluid mass to avoid getting cells which might have been protected by a thick layer of protein at the edges of the cell. The pour plates did not show immediate growth, but only after two or three days, suggesting slow recovery of injured cells or germination from spores. The number of cells present, as shown by dilution studies, was at most several hundred per cubic centimeter. The finding is cited mainly to indicate the extreme difficulty in sterilizing a 1% protein solution by irradiation, even when it is well stirred and not over 7 or 8 mm in thickness.

297. THE EFFECT OF THIAMIN DEFICIENCY UPON THE HISTOLOGY OF THE THYROID OF THE ALBINO MOUSE. Norma Vincent and J. Theron Illick, Syracuse University.

Albino mice fed vitamin B₁ free diet developed experimental beriberi, a pure thiamin deficient condition, which so far as could be determined was uncomplicated by any other factor. Histological changes occurred in the thyroid gland of these thiamin deficient mice indicating a state of quiescence of the thyroid during thiamin deficiency. These results are in agreement with the observations of other investigators on the thyroids of thiamin deficient rats.

298. ON THE PRESENCE IN THE SERA OF DOMESTIC FOWL OF NATURALLY-OCCURRING "AGGLUTININS" FOR CERTAIN MELANINS. James D. Ebert and S. H. Goodgal, The Johns Hopkins University. (Introduced by B. H. Willier.)

In an investigation of the antigenic properties of melanin pigments, normal sera of adult chickens, of 4 genotypes distinctive in pigmentation, were shown to agglutinate specifically black melanins obtained from three sources: (1) acid hydrolysis of Barred Plymouth Rock feathers, (2) untreated squid ink, and (3) auto-oxidation of 3,4, dihydroxyphenylalanine; yet these normal sera fail to react with "red melanins" extracted from Rhode Island Red feathers by acid or alkaline hydrolysis. Normal sera of rabbits and 8 other mammalian species fail to produce a similar agglutination.

The reaction occurs rapidly at room temperature; low temperatures do not markedly raise the titer, which is 1:32. There is no evidence of the titer rise in vitro characteristic of precipitins in immune fowl sera. The active agent is not destroyed by heating at 56°C. for 30 minutes; hence complement is not essential. Melanins are not agglutinated by serum which has been heated to 75°C. for 5 minutes; however they no longer react when resuspended in unheated serum, a fact which indicates the production of a "blocking" (univalent) form of the active substance. The substance is distinct from natural tissue antibodies. No increase in titer was produced by injections of dopa melanin, although in several cases the dosage totaled 150 mg of melanin. The characteristics of the reaction, and the inability to increase the titer by melanin injections suggest that the agglutination is due either to a true natural agglutinin or to the oxidative coagulation of non-globulin serum constituents.

299. CHANGE IN RATE OF GROWTH INITIATION ON SEASON. Frederick S. Hammett, The Lankenau Hospital Research Institute, Philadelphia, Pennsylvania.

The growth activity of initiation consists of shift in cell activities from state of being to state of growth. It is expressed in *Obelia* by buds coming: *a.* On the hydrocaulus wherefrom no hydranth or gonangium has before existed. *b.* On the terminal annulation of a pedicel on which a hydranth has previously existed and passed away; *i.* In normal course of life-cycle progression; or, *ii.* By injury. The function of *a.* is colony population growth. Of *b.* replacement of hydranths lost to the colony. Rate of initiation for population growth from April into September (7 years hydranth records; 6 of gonangial) starts high, drops sharply to a mid-June low, and stays there. The course is not that of season change in temperature, or any other environmental factor; nor are the week-to-week changes in rate positively correlated with those of temperature. Thus the course of change in rate is not due to season. Its basis is the increasing colony maturity which takes place as season advances. The rate of initiation for replacement of parts lost to the colony changes not in essence on season. It does not react either to week-to-week changes in temperature; nor to its season course of change. These facts show that the course of rate of this growth activity on season is not season-induced. But is a product of endogenous factors (colony maturing and repair). And that endogenous control is basically superior to exogenous influences. In other words, there is change on season which is not season factored.

300. THE ANTAGONISTIC ACTION OF ADRENAL CORTICAL EXTRACT ON THE INHIBITORY METABOLIC EFFECT OF LARGE DOSES OF SESAME OIL. Thomas B. Calhoun and Clifford A. Angerer, Department of Physiology, The Ohio State University, Columbus.

Oxygen consumption determinations were made on two groups of goldfish over a period of 8 weeks using the Winkler technique. These experiments were then checked against a new respirometer developed in this laboratory. All fish were fasted from 12 to 18 hours prior to use in order to render the animal in a "basal" state. A group of 12 fish were given 0.0036 cc of sesame oil/gram weight intramuscularly every other day for the 8 week period. Oxygen consumption determinations were made at the end of each week. At the end of the first week the mean metabolic rate had decreased 28.0% as compared with normal. The lowest (31.0%) metabolic rate occurred at the end of the 8th week.

A second group of 6 goldfish were injected in the same manner with 0.0036 cc of sesame oil and 0.00036 cc of Upjohn's adrenal cortical extract/gram weight. At the end of the first week the mean metabolic rate had risen 13.5%. This was followed by a slow decrease to 11.0% below normal at the 8th week. Statistical analysis (Student's method) shows that at the first week the percent difference between the two groups is 41.5%, $p = < .01$, and at the 8th week the percent difference is 20.0%, $p = < .03$. Four of the 6 fish given simultaneously sesame oil and adrenal cortical extract showed no weight gain during the 8 weeks. The other two fish gained more weight than those given sesame oil alone.

301. THE EFFECT OF X-IRRADIATION ON THE RESPIRATORY METABOLISM OF NON-NUCLEATED ERYTHROCYTES IN VITRO. Luis Angelone, Joseph L. Morton and Clifford A. Angerer, Department of Physiology and Department of Radiology, The Ohio State University, Columbus.

Since there is no literature which treats of the effects of x-irradiations on the respiratory metabolism of non-nucleated red cells in vitro, it becomes necessary to make such a study to extend previous metabolic studies on these cells (Gonzalez and Angerer, '47; Angelone and Angerer, '49). Erythrocytes obtained from 32 to 43 normal, male, white rats (100-150 gm) by means of cardiac punctures were pooled, washed twice, centrifuged to constant volume, resuspended in an equal volume of Krebs-phosphate Ringer solution (pH 7.4) and stored at 2° to 8°C. Suspensions were returned to room temperature for oxygenation and for withdrawal of aliquot samples for respiratory study by the Warburg technique. All experiments were paired (control and x-irradiated). Three pools of 10,000 r, one pool of 20,000 r, and three pools of 30,000 r, showed respectively a 20% to 42%, a 28%, and a 9.6% to 45% stimulating effect on the oxygen consumption as compared to the controls toward the end of 24 hours. There followed (second and third day) a decrease in oxygen consumption to and below that value of zero time (before x-irradiation). Since the average of the 30,000 r stimulation was far below that of the 10,000 r, one pool studied so as to accentuate the irradiation effect, showed a decrease of -37% as compared to the control at the end of 24 hours. This may have been fortuitous and therefore indicates further studies.

302. THE INTERACTION OF DILUTE AMYL CARBAMATE AND CERTAIN CATIONS ON THE RESTING POTENTIAL OF FROG NERVE. Frederick Crescitelli (with the technical assistance of Philip B. Hollander), Department of Zoology, University of California, Los Angeles.

Amyl carbamate at the proper concentration is capable of producing a small and reversible increase in the resting potential of frog nerve. Conversely, ions of potassium, rubidium, lithium and barium are known to be capable of reversibly depolarizing the same nerve. This paper is concerned with the interaction between amyl carbamate and these several ions, in modifying the nerve resting potential. The results may be summarized as follows:

1. Exposure of the nerve to the narcotic does not prevent the depolarization of nerve by these cations. There is, instead, a significantly greater rate of depolarization by K^+ and Rb^+ after carbamate action, but the rate of depolarization by Ba^{++} and Li^+ is not significantly affected by the amyl carbamate.
2. The facilitation of the K^+ depolarization by amyl carbamate occurs not only at 23.6°C. but also at 5.2°C.
3. The production of a positive swing in the potential in a frog nerve by amyl carbamate will take place even when the membrane is depolarized to a certain level by K^+ . There is evidence, however, to indicate that this carbamate action does not occur in a nerve depolarized beyond a given level by K^+ .

These results warrant the interpretation that though amyl carbamate and K^+ act at different steps in the chain of reactions which determine the potential, there is a modification of the membrane so that the response to one of these substances is modified by previous treatment of the nerve with the other.

303. QUANTITATIVE EVALUATION OF HISTOCHEMICAL PREPARATIONS FOR PHOSPHATASES. W. L. Doyle, University of Chicago.

Visual or photometric measurement of the sulfide precipitate on a tissue section prepared by Gomori procedures is an unreliable index of the amount of enzyme present. Fogo and Popowski, '49, employed para-aminodimethyl aniline sulfate plus ferric chloride and hydrochloric acid to estimate sulfide spectrophotometrically as methylene blue. This reaction is readily adaptable to Gomori histochemical preparations in which the sulfide is precipitated as lead sulfide. It is then possible to compare the total amount of precipitate in different sections and to determine objectively the enzymatic reaction rates of different preparations in the substrate mixtures. The method has been checked against independent quantitative procedures on adjacent sections and is reliable for phosphatase. It should be suitable for quantitative assay of other histochemical preparations yielding sulfides. Moderate sized sections with definitely blackend areas yield sufficient methylene blue for colorimetry of 5 ml samples.

304. EFFECT OF SALT CONCENTRATION ON HISTOCHEMICAL PHOSPHATASE PREPARATIONS. W. L. Doyle, University of Chicago.

Substrate solutions used for phosphatase determinations by the Gomori procedures have often been modified. Major consideration has been directed to the change in the hydrolysable component which is usually a sodium salt of a phosphate ester. With glycerophosphate substrates we have found that increases of 0.2 to 0.5 M in sodium chloride concentration produce marked diminution of intensity of acid and alkaline phosphatase staining reactions. The salt content also affects the solubility of the substance normally precipitated during the reaction. Added sodium chloride increases the solubility of calcium phosphate in the alkaline substrates and decreases the solubility of lead phosphate in the acid substrates. Independent quantitative measurements of the enzyme activities indicate (for rabbit lymphatic tissues) that alkaline phosphatase activity is unaffected by 0.2 M sodium chloride and only partially inhibited at 0.5 M. Acid phosphatase activity is unaffected by the added salt. Thus the inhibition of the Gomori staining reaction remains unexplained but it appears to be desirable when modifying substrates to control concomitant changes in ion content.

305. TUMOR MORTALITY AND SEX IN *LEUCOPHAEA MADERAE* (ORTHOPTERA).¹
Berta Scharrer,² University of Colorado, Medical Center.

Following severance of the recurrent nerve in *Leucophaea* more females die earlier from the resulting tumors than males. Thus, in a series (A) of 43 female tumor deaths, 32 (74.4%) occurred within 60 days after operation; 9 of these

animals died at intervals from two to 22 days. In the corresponding male series of 27 the earliest death occurred 23 days after operation; only 8 (30.7%) died within 60 days. Nine males and three females with tumors survived more than 150 days. The mean survival time in this series (A) was 56.2 days for tumor females and 113.3 days for tumor males. In a parallel series (B) of castrates with subsequent nerve severance this sexlinked difference was no longer observed. Of 42 tumor deaths in ovariectomized females, 40.4% were recorded within 60 days, while of 59 tumor deaths in castrate males 40.6% died within the same period. The mean survival rates in series B were 82.1 days for females and 81.2 days for males. Thus the survival rates in tumor bearing gonadectomized males and females are equal and represent values intermediate between males and females of series A. These differences may be correlated hypothetically with sex differences in metabolism. The greater lability in the metabolic pattern of the reproducing females may account for their lower resistance to tumor growth. (See abstr. no. II.)

¹ Work supported by the American Cancer Society and The Anna Fuller Fund.

² Fellow of the U. S. Public Health Service.

306. FAT METABOLISM IN TUMOR BEARING INSECTS (*LEUCOPHAEA MADERAE*, ORTHOPTERA).¹ Virginia T. Wilson and Berta Scharrer,² University of Colorado, Medical Center.

The sexlinked difference in mortality from tumors caused by nerve severance does not hold for previously gonadectomized animals (see Abstr. no. I). In order to correlate these observations with possible differences in metabolism, the fat content of whole animals was determined in relation to total dry matter and body weight. A comparison of the values for normal adults of known age, for starved, for castrate, and for tumor bearing animals showed significant differences. The content in body fat (3.34 to 13.7% of total dry matter) of the tumor bearing males examined (7 animals with cut recurrent nerves; 15 with nerves cut several weeks after gonadectomy) was below normal and close to starvation levels. The corresponding values for females showed a much wider range. In 13 tumor bearing females the fat content ranged from 5.09% to 26.5%, with 6 of the specimens showing values well within the normal range of adult females. Among 12 ovariectomized females with tumors, 2 showed values (about 40%) higher than normal and typical of castrates, 4 were within normal range, and 6 were below normal (minimum 6.99%). These preliminary data seem to indicate an essential difference between males and females. They also show that, at least among females, tumor deaths may occur at a time when the body reserves in fat are not yet diminished. In such animals death cannot, therefore, be attributed to starvation in connection with tumorous changes in the alimentary organs.

¹ Work supported by the American Cancer Society and The Anna Fuller Fund.

² Fellow of the U. S. Public Health Service.

307. THE ELECTRICAL RESPONSE TO ILLUMINATION OF THE MEDIAN OCELLUS OF LIMULUS. Verner J. Wulff, University of Illinois.

On the anterior, dorso-medial aspect of young *Limulus* two small ocelli may be seen, one on each side of a spine on the carapace. Each ocellus consists of a cluster of sense cells, pigment and supporting cells. These are located under a translucent area of the carapace, the lens and cornea. Each cluster of cells gives rise to a small nerve, which fuse to form a larger trunk, which descends around the esophagus. Near the junction of the two nerves there exists a white body which also gives rise to a small bundle of nerves. The nerve trunks, along with the clusters of sense cells are readily excised and drawn into a short oil-filled capillary tube. This tube is mounted in an electrode chamber so that the recording circuit included the sense cells and a portion of the attached nerve. Illumination of this preparation elicits transient electrical responses whose time course and magnitude resemble those obtained from the lateral eye. This preparation submits readily to surgical procedure. Removal of one cluster reduces the magnitude of the potential about one-third. Removal of both clusters of sense cells which constitute the ocelli and illumination of the remaining white body results in an electrical response similar to that obtained from the ocellus. Removal of all structures except a small part of one cluster and subsequent illumination results in a wavy electrical response quite distinct from the typically smooth contour. This uneven response suggests summation of electrical responses of individual units whose latencies are slightly variable.

308. HISTOLOGICAL CHANGES INDUCED IN THE RAT BY FEEDING ON ACID HYDROLIZED TRYPTOPHAN-DEFICIENT DIET. F. B. Adamstone, University of Illinois and Harry Spector, Quartermaster Food and Container Institute for the Armed Forces.

Using a force-feeding technique to prevent complications due to inanition, rats were fed an acid-hydrolyzed casein diet deficient in tryptophan. A very rapid response to the deficiency was obtained and definite histological reactions occurred. In the liver a progressively increasing infiltration of fat developed, accumulating first in the periportal areas of the lobules and gradually working to the center. By 16 days the entire liver was heavily loaded with fat. Accompanying this reaction the hepatic cells became highly vacuolated and the mitochondria increased greatly in size. A single large nucleolus was present in the nucleus rather than several which occur normally. The testes of the male underwent a series of degenerative changes marked by giant cell formation and sloughing of cellular elements of the tubules, the whole process being remarkably similar to that produced by Vitamin E deficiency. Atrophy and degeneration of cardiac muscle and also visceral muscle of the intestine and urinary bladder occurred as well as some injury to epithelial tissue of the bladder and keratinization of the cornea. Control animals did not develop these symptoms and tryptophan-deficient animals were cured by l-tryptophan therapy.

309. SOME ASPECTS OF BEHAVIOR OF OYSTERS ACCUSTOMED TO DIFFERENT SALINITIES. V. L. Loosanoff and P. B. Smith, U. S. Department of the Interior, Fish and Wildlife Service.

Using our apparatus by means of which it is possible to change the salinity gradually, imitating the changes occurring between the high and low water stages in many intertidal basins, observations were made on the pumping and shell movements of oysters accustomed to living in different salinities.

During a decrease in salinity, as on ebb tide, oysters taken directly from Long Island Sound, where the salinity is always approximately 27.0 parts per thousand (p.p.t.), stopped pumping at an average salinity of about 12.0 p.p.t. They often closed their shells before the salinity was reduced to about 8.0 p.p.t. and remained closed while the salinity continued to decrease further until the water became fresh. When, as on flood tide, the salinity began gradually to increase, the majority of the oysters opened their shells when it reached about 12.0 p.p.t. and began pumping again at about 13.5 p.p.t. However, wide variations in the behavior of different individuals were observed.

Long Island Sound oysters which were conditioned in the laboratory for about three months in a salinity of 10.0 p.p.t. ceased pumping water at an average salinity of about 4.0 p.p.t. and closed their shells at about 2.1 p.p.t. On increasing the salinity they opened their shells at about 4.5 p.p.t. and began pumping at about 5.0 p.p.t.

Oysters conditioned at 7.5 p.p.t. ceased pumping when the salinity was decreased to approximately 3.0 p.p.t. and closed their shells at about 1.1 p.p.t. When the salinity began to increase, as on flood tide, these oysters opened their shells at approximately 2.5 p.p.t. and began pumping at about 3.5 p.p.t.

These experiments showed that oysters can get accustomed to a much lower salinity than the one in which they were grown.

As long as the oysters remained open, the changes in the salinity of their shell and body fluids closely followed the changes in the salinity of the surrounding water. In general, the salinity of the sea water always remained somewhat higher than the shell fluid, while the body fluid showed the lowest salt content.

310. INCREASED INCIDENCE OF A TUMOR OF *DROSOPHILA* IN THE PRESENCE OF HIGH CONCENTRATIONS OF ARGININE.¹ Louise Palmer Wilson, Wellesley College.

Arginine in concentrations of 0.1 M and 0.05 M added to a sterile basal culture medium containing brewer's yeast increases the incidence of a melanotic tumor in *Drosophila melanogaster*. In this strain the tumor is found only in or on the fat body and begins to show melanin 97 to 102 hours after hatching. Incidence in the control larvae is highly variable, but, in all of 15 experiments flies fed arginine showed a higher incidence of tumors than did control flies from eggs laid by the same mass culture of parents and treated identically except for the addition of arginine. The percentage differences in 10 experiments vary from 20 to 59% more flies with tumors among the arginine-treated flies than among the controls. Five experiments, in which the incidence of tumors in the controls was much higher than in the first set, showed an increase from 6 to 22% more tumors among the experimental than in the control flies.

In arginine-treated larvae tumors are detected from 6 to 10 hours earlier than in larvae fed only the basal medium. In one test using basal medium, 100 flies with one or more tumors had 168 tumor sites while 100 such flies fed basal medium plus arginine showed 328 tumors of various sizes.

¹ Aided by a grant from The American Cancer Society.

311. EFFECT OF DECOMPRESSION ON THE GLUCOSE CONCENTRATION OF THE BLOOD OF THE FROG, *RANA PIPPIENS*. Robert K. Houlihan and John J. Petronio, Boston College.

Preliminary investigations indicate that effects on living systems, particularly on the amount of blood sugar, brought about by reduced pressures are dependent on more than one factor. The animal used was *Rana pipiens*.

Four series of tests were carried out during which the amount of air and the rate of decompression was varied. In all cases the frogs were brought to, and maintained at, a simulated altitude of 60,000 feet for one hour. The terms fast decompression and slow decompression are referred to in this paper and mean decompression rates of 2000 feet per second and 250 feet per second respectively.

In the first test, fast decompression without air, the amount of glucose in the blood decreased by 12.14%. In the second test, fast decompression with an air supply, the frogs showed a 42.15% increase in blood sugar. In the third test, slow decompression without air and slow decompression with air, there were blood sugar increases of 83.70% and 59.28% respectively.

When the results of tests conducted under anoxic conditions are compared with those in which air was supplied, a definite difference is noted indicating that the amount of air available is an important factor. Also when the changes which occurred from different rates of decompression are compared another difference is noted. Thus the rate of decompression is also important. So it seems that the blood sugar level during decompression depends upon a combination of factors. Other factors being investigated now are the degree of decompression and the length of time of exposure to reduced pressures.

Parasitology

312. PARASITES OF THE RANIDAE (AMPHIBIA). XV. A. C. Walton, Knox College.

The annotated catalog of the parasites of the genus *Rana* continues the list of hosts and their parasites as follows: 67. *Rana nutti* (Africa) — *Amplichaecum involutum* (Nematoda); and *Balantidium* sp? of Metcalf ('23), *Haemogregarina* spp? of Averintsev ('13), and of França ('17), *Nyctotherus amaniensis*, *N.* sp? of Metcalf ('23), *Protoopalina nutti*, *P. primordialis*, *Pseudogregarina ranae*, *Trypanosoma karyozeukton*, *T. rotatorium*, and *T. tumida* (Protozoa). 68. *Rana occipitalis* (Africa) — "Microfilaria" sp? of Schwetz ('30) (Nematoda); and *Haemogregarina* sp? of Schwetz ('38), *Lankesterella* sp? of Schwetz ('38), and *Trypanosoma rotatorium* (Protozoa). 69. *Rana oxyrhynchus* (Africa) — *Haemogregarina pestanae*, *Toodia* sp? of França ('17), *Trypanosoma rotatorium*, and *T.* spp? of Dutton, et al. ('07), and of Laveran and Mesnil ('12) (Protozoa). 69a. *Rana oxy-*

rhynchus gribinguiensis (Africa) — larval *Physaloptera* sp? of Loveridge ('36) (Nematoda). 70. *Rana palmipes* (Venezuela) — *Glythelmins palmipedis*, *Gorgoderina parvicava*, *Haematoloechus iturbei* (n. comb. for *Pneumonoeces iturbei* Cordero and Vogelsang ['39]), *H. neivai*, *H. tejerae* (n. comb. for *P. tejerae* C. and V., '39), larval *Lophosicyadiplostomum nephrocystis*, and larval *Neodiplostomum branchiocystis* (Trematoda). 71. *Rana palustris* (U.S.A.) — *Capillaria tenua*, *Cosmocercoides dukae*, *Oswaldocruzia collaris* (♀ only), *O. leidyi*, *O. pipiens*, *Oxy-somatium americana*, ? *Rhabdias bufonis*, *R. entomelas*, and *R. ranae* (Nematoda); and larval *Alaria mustelae* (in tadpoles and in adults), *Brachycoelium salamandrae*, larval *Clinostomum complanatum*, larval *Euryhelmis monorchis* (in tadpoles and in adults), ? *Gorgodera cygnoides*, *Gorgoderina attenuata*, gorgoderid cysts of Rankin ('45), *Haematoloechus medioplexus*, and ? *Haplometra cylindracea* (Trematoda).

313. PARASITES OF THE RANIDAE (AMPHIBIA). XVI. A. C. Walton, Knox College.

71 (con.). *Rana palustris* (U.S.A.) — *Amphileptus branchiarum* (on tadpoles), *Balantidium falciformis*, *Epistylis* sp? of Wenrich ('35) (on tadpoles), *Glossatella tintinnabulum*, *Haptophrya virginienensis*, *Ichthyobodo necatrix*, *Lankesterella* sp? of Scott ('26), *Nyctotherus parvus*, *Opalina larvarum* (in tadpoles), *O. obtrigonoidea* (in tadpoles and in adults), *O. obtrig. plicata*, ? *O. triangularis*, *Opercularia* sp? of Wenrich ('35) (on tadpoles), *Rhabdostyla* sp? of Wenrich ('35) (on tadpoles), *Trichodina* sp? of Wenrich ('24) (on tadpoles), *Trichomonas augusta*, and *Vorticella* sp? of Wenrich ('35) (on tadpoles) (Protozoa). 72. *Rana pipiens* (N. Amer.) — (published elsewhere). 73. *Rana planeyi* (China) — *Rhabdias incerta* (Nematoda); *Haematoloechus nanchangensis* (Trematoda); and *Protoopalina pingi*, *P. quadrinucleata*, and *Trypanosoma* sp? of Ogawa and Uegaki ('25) (Protozoa). 74. *Rana pretiosa* (U.S.A.) — *Aplectana gigantica*, and *Spiro-noura pretiosa* (Nematoda); *Gorgoderina multilobata*, *G. tanneri*, *Haplometrina intestinalis*, and *H. utahensis* (metacercaria and adults) (Trematoda); and *Opalina copei* (Protozoa). 75. *Rana rugosa* (Japan) — *Cosmocerca japonica*, *Gyrinicola japonica* (in tadpoles), *Hedruris ijimae*, *Oswaldocruzia filiformis* (the *O. insulae* of Morishita ['26]), *Rhabdias bufonis*, *R. nipponica*, and *Spiroxys japonica* (Nematoda); *Diplodiscus amphichrus* (also from Korea), *D. japonicus*, *Diplorchis ranae*, larval *Echinostoma cinetorchis* (in tadpoles), *Glythelmins rugocaudata*, *Loxogenes liberum*, *Mesocoelium brevicacum*, *M. elongatum*, *M. ovatum*, and *Opisthodiscus subclavatus* (Trematoda); *Acanthocephalus nanus*, and larval *Centrorhynchus* sp? of Van Cleave ('25) (Acanthocephala); and *Cepedea pulchra japonica*, and *Trypanosoma* spp? of Laveran and Mesnil ('12), and of Koidzumi ('11) (Protozoa).

314. PARASITES OF THE RANIDAE (AMPHIBIA). XVII. A. C. Walton, Knox College.

76. *Rana rugulosa* (China, India, Siam) — *Gnathostoma spinigerum* (Nematoda); ? larval *Centrocestus formosanus*, *Diplodiscus amphichrus*, *D. japonicus*, *Ganeo lingnanensis*, *Glythelmins staffordi*, larval *Pharyngostomum cordatum* (in tadpoles and in adults), and *Pleurogenes taylora* (Trematoda); larval *Diphyllbothrium erinacei*, and *Proteocephalus tigrinus* (Cestoda); and *Balantidium ranarum*, *B. sushilii*, *B.* sp? of Chakravorti ('33), *Nyctotherus magnus malabarica*,

N. piscicola (normally in fish), and *Trichomonas batrachorum* (Protozoa). 77. *Rana septentrionalis* (Canada) — *Opalina* sp? of Metcalf ('23) (Protozoa). 78. *Rana similis* (Philippines) — *Cepedea luzonensis* (Protozoa). 79. *Rana sphenocephala* (U.S.A.) — *Abbreviata ranae*, *Cosmocercoides dukae*, *Foleyella americana*, *F. brachyoptera*, *F. dolichoptera*, *F. leiperi*, *F. ranae*, *F. sp?* of Causey ('39), larval *Multicaecum tenuicolle*, larval *Ophidascaris labiatopapillosa*, *Oswaldocruzia collaris*, *O. pipiens*, *Rhabdias entomelas*, *R. ranae*, and *Spironoura catesbianae* (Nematoda); larval amphistome of Trowbridge and Hefley ('34), *Brachycoelium salamandrae*, larval *Clinostomum complanatum*, *Glypthelmins quieta*, *G. subtropica*, *Gorgodera amplicava*, *G. cygnoides*, *Haematoloechus uniplexus*, *Megalodiscus temperatus*, and larval *Neodiplostomum* sp? of Penner ('38) (Trematoda); *Ophiotaenia magna*, and proteocephalid cysts of Brandt ('36) (Cestoda); larval *Centrorhynchus* sp? of Brandt ('36) (Acanthocephala); *Cytamoeba bacterifera*, *Entamoeba ranarum*, *Hexamita intestinalis*, *Karyolysus* sp? of Brand ('36), *Lankesterella* sp? of Brandt ('36) *Leptotheca ohlmacheri*, ? *Nyctotherus cordiformis*, *Opalina carolinensis*, *O. kennecotti*, *O. obtrigonoidea* (? = *O. triangularis* Ghosh ['18]), *O. sp?* of Brandt ('36) *Trichomonas augusta*, and *Trypanosoma rotatorium* (Protozoa); *Aëdes aegypti*, *A. atropalpus*, *Culex apicalis*, *C. fatigans*, *C. pipiens*, and *C. quinquefasciatus* (Diptera); and larval mite of Hubert ('27), *Hanne mania eltoni*, and *H. penetrans* (Arachnida).

315. THE EFFECTS OF X-RAY ON RHABDITIS SPECIES. Lyell J. Thomas, University of Illinois.

Rhabditis species, a nematode producing scabies in dairy cattle, was x-rayed with 10,000, 20,000, and 40,000 Roentgen units. The first generation larvae showed accelerated mutation in the production of differences in refractive indices of sections of the intestine containing Rhabditin granules. The nematode may be dried for as long as 9 months and be revived. X-ray effects in the dry state required twice as much to produce the same effect as in the wet state.

Protozoology

316. THE USE OF THE UROID AS A TAXONOMIC CRITERION FOR CERTAIN AMEBAS.

Eugene C. Bovee, University of California at Los Angeles. (Introduced by Theodore L. Jahn.)

By the employment of the phase microscope it has been determined that most naked lobose amebas form some sort of trailing filaments at the posterior end. These are formed from hyaline ectoplasm by stretching it into filaments as it is pulled away from adhesive contact with the substrate as the posterior end of the ameba passes the point of adhesion. In those organisms where the posterior end is permanently located at a heavily gelated portion of the organism, the uroid is not quickly reincorporated with the general ectoplasm, but trails behind the organism as a tuft attached to the periphery or general surface of the permanent posterior area. This permanent site of uroidal formation is commonly present in the genera *Pelomyxa* (except with the controversial *Chaos* [*Pelomyxa*] *carolinensis* Wilson) and *Trichamoeba*. There is no permanent posterior region in the repre-

sentatives of the genera *Chaos*, *Metachaos*, or *Polychaos*, nor in any of the Mayorellidae which have been examined to date. It is suggested that permanency of the site of the uroid may be used as a generic character, and that the details of its form may be used frequently in the determination of the species.

317. SOME OBSERVATIONS CONCERNING THE MORPHOLOGY, LOCOMOTION, AND RATE OF ACTIVITY OF *TRICHAMOEBA OSSEOSACCUS* (SCHAEFFER). Eugene C. Bovee, University of California at Los Angeles. (Introduced by Theodore L. Jahn.)

As described by Schaeffer ('26), the genus *Trichamoeba* does not exhibit a stellate stage. These observations, made with numerous organisms over a period of several weeks, indicate that the usual condition for *T. osseosaccus* when disturbed is a well-defined and relatively long-continued stellate form. In slow locomotion, particularly in debris, it employs digitate pseudopods resembling those of *Metachaos discoides*. The clavate form is predominant in rapid locomotion. It has been determined by following numerous organisms from rest through all phases of locomotion and back to rest, that *T. osseosaccus* possesses a permanent posterior area of a discoid shape at the ventral periphery of which the uroidal filaments form. The uroidal filaments are entirely ectoplasmic in origin. This organism appears to possess a definite diurnal rhythm, during which the external form exhibited correlates roughly with the hour of the day. The stellate form is predominant in the early morning hours; the pseudo-metachaos form in mid-morning; the clavate form in mid-day and early afternoon; the pseudo-metachaos form in mid-afternoon; and the stellate form in late afternoon. These changes are relatively unaffected by continued exposure to light and heat from the microscope lamp.

318. OBSERVATIONS ON *EIMERIA MOHAVENSIS* SP. NOV. FROM THE KANGAROO RAT *DIPDOMYS MOHAVENSIS*. David J. Doran and Theodore L. Jahn, University of California at Los Angeles.

Eimeria mohavensis, sp. nov., is described from *Dipodomys mohavensis*. Size: 21.5 μ to 26.0 μ , average of 24.1 μ , by 14.0 μ to 18.5 μ , average of 15.7 μ . Schizogonous forms are similar to those of other species of *Eimeria*. Sporogony is irregular and results in a diversity of intermediate segmentation stages. When taking as a starting point the placing of immature oocysts in 2-4% potassium dichromate, and observing the time of beginning segmentation and the time of constant percentage of completely sporulated oocysts, the sporulation time is from 24-64 hours.

The prepatent period is 8-9 days. The patent period is 9-10 days. Rats fed rolled barley seemed to have a shorter patent period than those fed rolled oats.

Rats fed rolled barley, deficient in vitamins B and G, produced one-half to one-third as many oocysts as rats fed rolled oats.

Reinfection of an animal that had ceased to produce oocysts 10 days previously was successful. However, oocyst production was below that of the initial infection.

Oocysts produced during initial infection do not vary in average length from those produced on reinfection.

There is no variation in average oocyst length during the patent period. However, the range of length of oocysts produced during the third and 4th days is greater than those produced at the beginning and end of the patent period.

Juvenile *D. mohavensis* produce oocysts having a greater average length than do adults.

There is no difference in average length of oocysts produced during light infection and heavy infection.

319. STUDIES OF AMPHIBIAN TRICHOMONADS IN CULTURE. Robert Samuels, University of California at Berkeley. (Introduced by Harold Kirby.)

Continuous, single-tube culture of *Tritrichomonas augusta*, *T. batrachorum*, and *Trichomonas prowazeki* reveals many interesting phenomena.

Ingestion of starch grains occurs anywhere on the body surface through temporary food cups. Observations of ingestion through the cytostome are lacking. Periodically, cannibalism is common. In living material, both organisms are active in the process. First, both extend broad pseudopodia by which they adhere. The ingestor finally forms a food cup and encompasses the other in a food vacuole. Permanent preparations show the formation of food tubes like those of *Entamoeba* and *Histomonas* accompanying intake and breakup of the ingested flagellate.

Morphological variations are common. Axostylar duplication occurs. (In *T. batrachorum*, the pelta is revealed to be part of a calyx cupping the nucleus and blepharoplast.) Occasionally, an extra flagellum appears. A normal blepharoplast may bear this, or a second blepharoplast well removed from that bearing the full mastigont system. Nuclear variations are especially frequent. Two or more nuclei may be present and, sometimes, the extra nucleus appears to come from an ingested trichomonad. Large, amoeboid nuclei with extra nucleoli concur with aneuploid and polyploid mitotic figures. Multipolar, irregular nuclear divisions may give rise to organisms with several unequal nuclei. Some division figures suggest meiosis, and evidence for nuclear fusion exists.

Integrating these nuclear variations suggests sexual phenomena as the source of some. The process, if it does occur, is still too unclear to permit a more definite statement. The study of mastigont variations may be useful in investigating morphogenesis.

320. THE EFFECTS OF COLCHICINE ON A FLAGELLATE PROTOZOAN, *TRICHOMONAS AUGUSTA*. Robert Samuels, University of California¹ at Berkeley. (Introduced by Harold Kirby.)

Trichomonas augusta was used to investigate the mitosis inhibiting effects of colchicine in a protozoan. Tests were performed on natural rectal infections of frogs by introducing a colchicine solution per anum.

The results obtained parallel those in higher organisms. During late prophase, the parasomes (central spindle) breaks down and the mitotic process is disrupted. The chromosomes condensing within the intact nuclear membrane retain fibrillar connections with the centriole. Later, organelles of the mastigont system are duplicated normally. The nucleus may follow several courses. It often wanders from the vicinity of the centrioles. It may retain the full complement of swollen, heavily condensed chromosomes, or constrict off vesicles containing one or two of the 6 chromosomes. Stages occur wherein the nucleus appears to be returning to

the interphase condition within a bimastigont organism. The nucleus remains with one blepharoplast-centriole complex; the other mastigont system and a portion of cytoplasm detach. No polyploid division figures were observed, but organisms with enlarged nuclei containing two nucleoli suggest ploidy may exist.

Selective destruction of the paradesmose indicates its homology with the central spindle of other mitoses. Normal reproduction of the mastigont systems after breakdown of the mitotic process suggests a possible origin of polymonads. If further research reveals aneuploidy and polyploidy, a possible tool exists for investigating heredity in a form in which a sexual cycle has not been demonstrated.

¹The author began this work while a Harrison Scholar at the University of Pennsylvania.

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EFFECT OF THYROID SUBSTANCES IN THE OVARIAN CAPSULE UPON MITOSIS IN THE GERMINAL EPITHELIUM

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SEVEN FIGURES

INTRODUCTION

In an earlier investigation (Stein, Quimby and Moeller, '47) it was reported that mitotic division in the germinal epithelium of both mature and immature mouse ovaries was retarded by thyroxine injected into the ovarian capsule. The retardation was considered to be due to a localized effect on the cells in question. A study of the pertinent literature concerned with direct effects of thyroid preparations on unicellular animals, in tissue cultures, and on early cleavage stages of invertebrates (see review by Schneider, '39) indicated that thyroxine usually suppressed or retarded cell multiplication, but that whole thyroid, on the other hand, had a stimulating effect. It was noted also that other factors such as dosage, route of administration, physiological condition of the tissue, or stage of differentiation, might be implicated in variations in response to thyroid preparations. The following experiments were undertaken, therefore, to determine whether the retarding effect on proliferation of the germinal epithelium reported previously was the result of an inherent difference in the activity of thyroxine from that of whole thyroid preparations or whether some other factor was involved.

MATERIAL AND METHODS

The mice used were albinos and offspring of several hybrid generations of a cross between the C3H and C57 strains. Some were immature but the majority were young adults weighing 20–30 gm. All were fed Pratt's Nurishmix and/or Purina dog chow supplemented with occasional milk and wheat germ.

One ovary of each animal served as the experimental ovary and was treated with a preparation of thyroid substance, or iodinated casein, usually diluted with Ringer's solution: the capsule around the other (control) ovary was injected with a similar preparation lacking thyroid. The operation to expose the ovaries for injection was performed under Nembutal anesthesia (0.2 cm³ of a 5% solution per 20 gm of body weight intraperitoneally) supplemented with ether. Injections were made through the fatty tissue of the capsule with a no. 26 needle attached to a hypodermic syringe. Enough fluid was injected to distend the capsule but care was taken to prevent rupture and as far as possible to avoid leakage. The amount of injected material could not be accurately controlled but was estimated to be less than 0.1 cm³ in all cases. Animals were sacrificed 24–28 hours after operation. To facilitate counting the mitoses colchicine in distilled water (0.0025 mg per gram body weight) was injected intraperitoneally 8–10 hours prior to killing. In most cases vaginal smears were taken to determine the stage of the estrous cycle at the time of operation and at death.

Care was taken to keep the capsule intact when the tissue was fixed. Fixers were Zenker-formol or Bouin's fluid and were followed by staining with Mallory's triple connective tissue stain or Mayer's hematoxylin and eosin respectively. All sections were cut at 10 μ . Mitoses were counted in every 5th section in series F and H, and the results multiplied by 5. This was found to give approximately the same total number as when every section was counted, the procedure followed in all other series.

PREPARATION OF INJECTED SUBSTANCES

Suspension of desiccated thyroid. Four grams of Armour's thyroid powder were added to 100 cm³ distilled water and 10 drops of 4% NaOH. The powder did not go into solution but remained in particulate form. The lack of any positive results from injection of this substance suggested that some treatment might be necessary to make the thyroid active or soluble and therefore capable of absorption by the cells. Accordingly the following preparations were made and used for other series of injections.¹

Digested thyroid powder. Harington and Salter ('30) have demonstrated that the thyroxine-containing fraction of an enzymatic hydrolysate of whole thyroid powder can be precipitated out at pH 5 and redissolved in alkaline solutions. In this manner they separated the thyroxine-containing fraction from the diiodotyrosine fraction after pepsin digestion, and the thyroxine fraction from certain other impurities after trypsin digestion and extraction. A peptone, corresponding to their first fraction, and a peptide, to their second, were prepared as follows. *Thyroid peptone.* Five grams of thyroid powder were mixed with 50 cm³ distilled water, 0.25 gm pepsin, and 5 cm³ 1N HCl. After 36 hours another 0.25 gm of pepsin and 5 cm³ HCl were added. Digestion was stopped, at 108 hours in series B2 and at 72 hours in series D and E, by bringing the pH to 5 with 1N NaOH. The precipitate, containing the thyroxine fraction plus broken amino-acid chains, was centrifuged out, dissolved in 25 cm³ 1N NaOH, and the pH brought to 7 with 25 cm³ 1N HCl. *Thyroid peptide.* Twenty-five cubic centimeters of the peptone solution was adjusted to a pH of 8 with NaOH and 0.5 gm trypsin was added. The solution was then incubated at 40°C. for 72 hours with readjustment of the pH to 8 twice during each day. Another 0.5 gm trypsin was then added and the solution incubated 24 hours. Digestion was arrested by bringing the pH to 5 with 1N HCl, the precipitate was centri-

¹ For assistance in the chemical aspects of the problem the authors are indebted to Associate Professor Jytte Muus of the Physiology Department.

fuged out, dissolved in 15 cm³ 1N NaOH and the pH brought to 7. This solution differed from the peptone in that it probably contained smaller chains of amino acids.

Iodinated casein. Since iodinated proteins are known to be active in the treatment of human thyroid deficiencies (Salter and Lerman, '38; Lerman and Salter, '39) a preparation of iodinated casein was made and used for injection. The method was a modification of that employed in the iodination of serum albumin by Muus, Coons and Salter ('41). Casein, which contains 6.4% tyrosine, was iodinated to put two atoms of iodine on every molecule of tyrosine. Five grams of casein were dissolved in 49 cm³ concentrated NH₄OH and 7.9 cm³ of 1N iodine in 25% KI added slowly. The reaction mixture was allowed to stand 15 minutes, neutralized with aqueous HCl (one volume concentrated acid to one volume distilled water) and the pH adjusted to 5. After standing 24 hours the acid insoluble brown precipitate was separated by centrifuging, dissolved in 1N NaOH and neutralized to pH 7.

Ringer's solution, casein dissolved in sodium hydroxide and neutralized with HCl, and casein digested with pepsin as in the preparation of thyroid peptone, were used for injection into the capsules of control ovaries (table 1,¹).

OBSERVATIONS AND RESULTS

In the two series of animals injected with a suspension of desiccated thyroid no positive results were obtained (table 1). This may have been due to the failure of the thyroid powder to dissolve. Differences in numbers of mitoses in the germinal epithelium between the two ovaries within each individual were not correlated with any known differences in the quantity or quality of injected material. The ovaries all appeared normal; and the average numbers of mitoses, 97 and 90, are very close to the average of 85 obtained by Stein and Allen ('42) in 11 control mice.

TABLE 1

Effect of thyroid preparations on mitotic division in the germinal epithelium

SERIES	NO.	WEIGHT IN GM	STAGE OF REPRO. CYCLE	NO. OF MITOSES IN THE G. E.		% INC. OF EXP.
				Control ¹	Experimental	
<i>Thyroid suspension</i>						
A	3	30.0	not. det.	141	137	
	5	23.0		180	112	
	6	18.5		35	73	
B ₁	1	21.7		52	65	
	2	27.0		82	48	
	3	26		92	105	
Average				97	90	0
<i>Thyroid peptone</i>						
B ₂	7	24.2	estrus	122	644	
	8	28.4	diestrus	70	464	
	9	28.4	estrus	77	150	
	10	26.4	diestrus	80	344	
D	1	13.0	immature	176	111	
	2	14.3	immature	125 ²	608	
	3	13.0	immature	41	87	
	4	13.4	immature	165	474	
	5	18.2	immature	495	279	
	7	15.6	immature	120	105	
	8	12.4	immature	187	301	
	10	15.6	immature	230	339	
	11	15.6	immature	230	175	
	12	19.0	immature	35	81	
E	1	21.5	estrus	170 ²	250	
	2	22.3	diestrus	160	175	
	3	20.0	diestrus	115	225	
	4	20.2	diestrus	100	375	
	5	18.5	no cells	165	295	
	7	20.7	vaginal plug	160	295	
	12	26.0	vaginal plug	320	275	
Average				159.2	288.2	81.1
<i>Thyroid peptide</i>						
F	1	20.5	estrus-di	135	465	
	2	22.8	estrus	240	465	
	3	21.0	estrus-di	100	475	
	4	22.2	estrus-di	250	120	
	5	19.4	estrus-di	150	180	
	6	21.2	estrus	185	415	
	7	23.3	estrus	150	395	
Average				172.9	359.3	107.8

TABLE 1 (continued)

SERIES	NO.	WEIGHT IN GM	STAGE OF REPRO. CYCLE	NO. OF MITOSES IN THE G. E.		% INC. OF EXP.
				Control ¹	Experimental	
<i>Iodinated casein</i>						
H	1	20.0	diestrus	90	575	
	2	20.9	met-di	125	330	
	3	24.4	estrus-di	90	75	
	4	20.0	early preg.	140	685	
	5	24.8	estrus	210	205	
	6	33.0	not det.	100	70	
	8	24.3	estrus	200	675	
	9	14.7	immature	235	445	
	Average			148.75	382.5	157.1

¹ Control ovaries of A, B₁, B₂, D and F were injected with Ringer's solution, E with pepsin-digested casein, H with dissolved casein.

² Estimated figure, some sections lost.

The products resulting from both pepsin and pepsin and trypsin digestion of thyroid powder stimulated mitotic proliferation in the germinal epithelium (table 1; figs. 1, 2, 4). The increase over the control ovaries treated with Ringer's solution or digested casein was 81.1% for the peptone and 107.8% for the peptide. Although the average results appear to indicate greater activity of the peptide the two experimental ovaries with the highest individual counts were peptone-injected (B-7 and D-2). Possible leakage after the return of the ovaries to the body cavity may be a factor in the lower average for the peptone series since it is the only explanation which can be suggested for the low counts in the experimental ovaries of animals D-7, D-11 and E-2.

It was also noted at the time of operation that in one animal (D-1) the ovaries were not well injected because of their small size. The low counts for both experimental and control ovaries in three animals (B-9, D-3, and D-12) is probably due to a lack of colchicine effect as indicated by the presence of very few mitoses in the follicles of these ovaries. There is a possibility that both ovaries were treated with thyroid in two animals, D-5 and E-12. In the peptide series the counts for individual ovaries are more consistent

than is true for the peptone and there are only two experimental ovaries (F-4 and 5) with low counts possibly due to leakage.

Iodinated casein produced the highest percentage of stimulation and also the highest individual counts (H-4 and 8) of all treated ovaries. The increase of 157.1% over controls

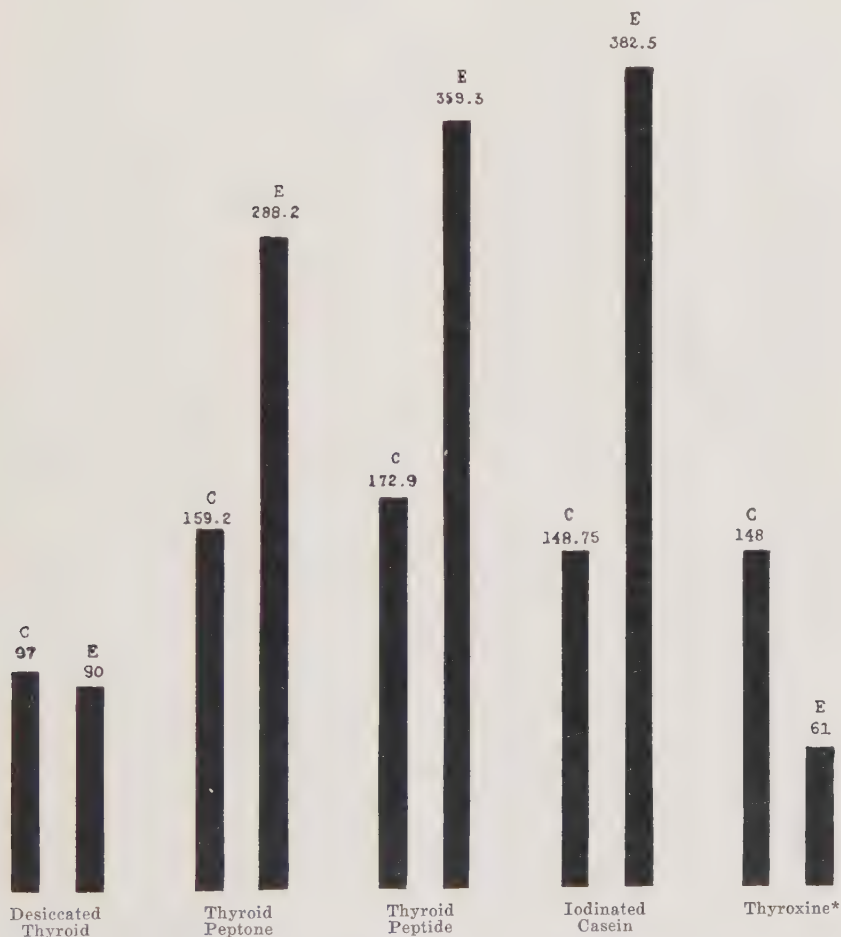


Fig. 1 Graph showing the effects of thyroid preparations on the germinal epithelium of the mouse ovary. Numerals give average numbers of mitoses for animals of each series. C, control, E, experimental. *Data from Stein et al. ('47).

includes three animals in which no effect was apparent in the experimental ovary. No explanation except possible leakage after injection can be offered.

DISCUSSION

Stimulation of mitotic division by digested thyroid preparations and by iodinated casein has been demonstrated in cells of the germinal epithelium by the experiments reported. The inactivity of whole thyroid powder is considered due to its insolubility, which might interfere with its access to the capsule through the small bore injection needle, or with its absorption by the cells. The stimulation resulting from the other preparations is believed to be a direct effect on the cells concerned, since it occurred in a short period of time after treatment, and since the effect in each animal was observed in most cases only in the one ovary treated with the digested thyroid or iodinated casein. In the other ovary, whose capsule was injected with a control solution, there was no apparent stimulation except in a few cases attributed to faulty technique or to the very slight possibility of a systemic effect.

It is believed that the substance directly responsible for stimulation from all three preparations was probably thyroxine in combination with amino acids. Thyroxine is known to be the active principle in desiccated thyroid, from which the digested preparations were made, and has been isolated from the latter (Kendall, '15) and also from iodinated casein and serum albumin (Ludwig and Mutzenbecher, '39; Harington and Pitt Rivers, '39; Reinicke and Turner, '43).

Of especial interest, however, is a comparison of the stimulation reported above with the apparent retardation of mitotic division produced in the same cells after capsular injections of pure crystalline thyroxine (Stein et al., '47). This result is in line with observations on the direct action of thyroid substances on mitosis in tissue cultures and in ex-

periments with protozoa and segmenting eggs of invertebrates (see review by Schneider, '39). Pure thyroxine, in general, depressed cell division in these forms while whole thyroid increased the number of dividing cells. Recent textbooks state that "thyroglobulin stimulates O_2 usage of tissues in vitro while thyroxine does not" (Everett, '46) and that "while thyroxine increases O_2 consumption given to the whole animal it does not have such an action upon isolated tissues" (Houssay, '46).

So far no explanation can be offered for the depression of mitotic division by thyroxine. Inactivity of the pure hormone acting directly on cells versus its activity when fed or injected, and versus the direct cellular effect of digested thyroid powder and iodinated casein, is reconcilable on the basis that, in the whole animal thyroxine is converted into an active form by the addition of amino acids. Kendall ('31) suggested that the functioning form of the thyroid hormone probably contained other amino acids and there is abundant evidence that whole thyroid, or thyroxine in preparations which contain other amino acids, is more active than pure hormone (Harington and Salter, '30; Harington, '33; Means, Lerman and Salter, '33; Lerman and Salter, '34; Salter, Lerman and Means, '33, '35; Meyer and Wertz, '39; Salter, '40; Craig and Salter, '45). It has been pointed out by Salter ('40) and reemphasized by Soskin and Levine ('44) that neither free thyroxine nor thyreoglobulin represent the circulating thyroid hormone. These substances can be extracted from blood but do not exist there free but rather in combination with other proteins. Harington ('33) suggested that thyroxine should be regarded as a constituent of a more active, probably a peptide, molecule. This idea is supported by Salter ('40).

The present study presents further evidence for the activity of thyroxine in combination with other amino acids. Digested thyroid preparations and iodinated casein stimulated mitotic activity in the germinal epithelium. Together with the previous report concerning the depressing effect of

thyroxine on the same tissue (Stein et al., '47) it supports the contention that pure thyroxine is not the active functioning form of the thyroid hormone.

SUMMARY AND CONCLUSIONS

1. Desiccated thyroid in suspension, pepsin and pepsin-trypsin digested thyroid powder (peptone and peptide respectively), and iodinated casein in solution were injected into the ovarian capsules of mice. One ovary of each individual was experimentally treated; the capsule of the other (control) ovary was injected with a solution lacking thyroid.

2. The desiccated whole gland in suspension produced no effect on the treated ovaries. This was considered to be due to its failure to dissolve.

3. The other substances mentioned all caused a definite stimulation of mitotic proliferation in the cells of the germinal epithelium.

4. This stimulation, compared with the previously recorded retardation of cell division caused by thyroxine, is considered to support the contention that the active functioning form of the thyroid hormone is not pure thyroxine but thyroxine in combination with other amino acids.

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PLATE 1

EXPLANATION OF FIGURES

Photomicrographs showing stimulating effect of thyroid substances in the ovarian capsule upon mitosis in the germinal epithelium. Ten micra. Bouin's fluid. Hematoxylin and eosin. $\times 260$.

2 Section of ovary of mouse B-7. Thyroid peptone injected into ovarian capsule.

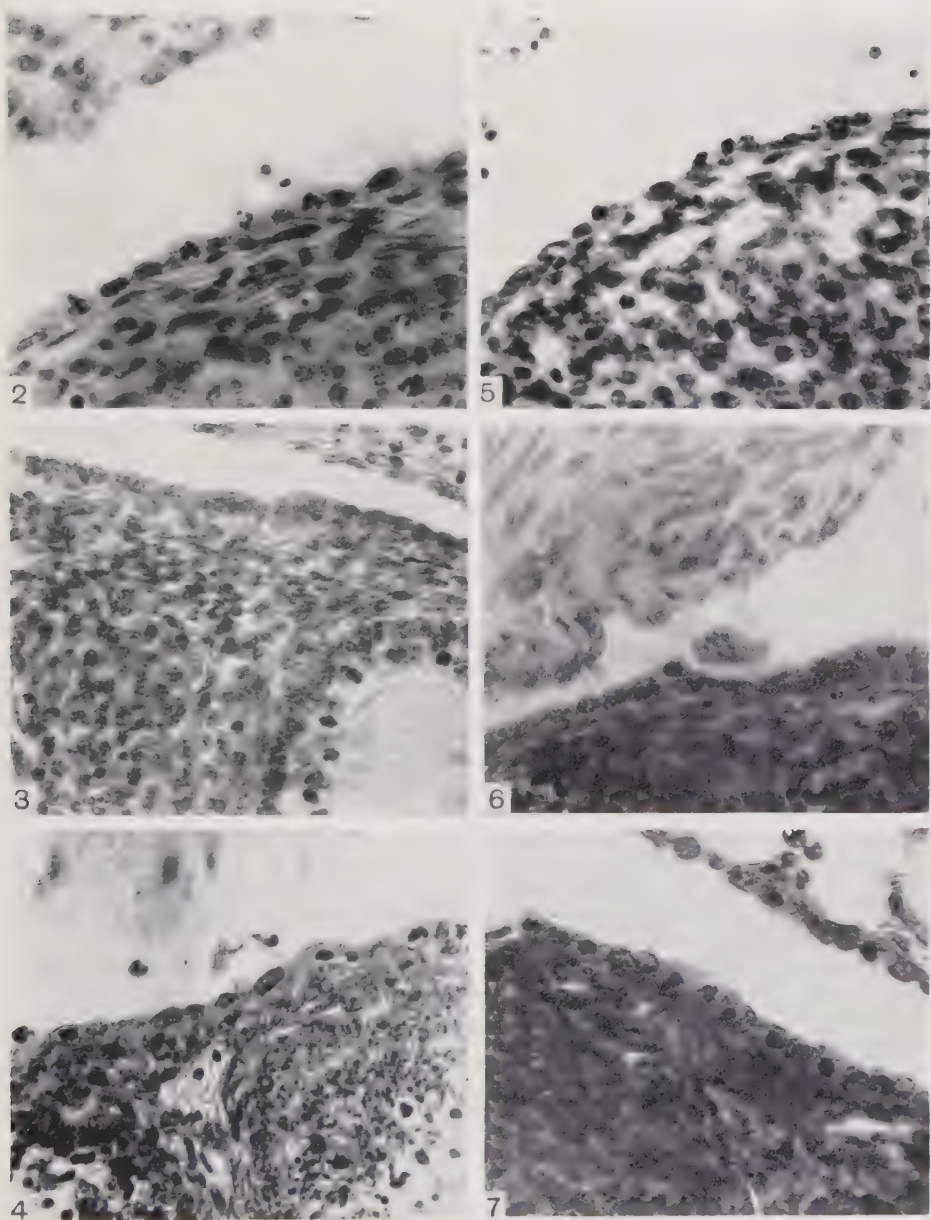
3 Section of control ovary of B-7. Ringer's solution.

4 Section of ovary of mouse D-2. Thyroid peptone.

5 Experimental ovary of mouse F-1. Thyroid peptide.

6 Control ovary of F-1. Ringer's solution.

7 Experimental ovary of mouse H-7. Iodinated casein.



SUPERFETATION IN A MOUSE¹

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THREE FIGURES

Superfetation is a rare phenomenon. Its occurrence in man is commonly refuted, since the action of pituitary hormones is supposed to suppress ovulation during pregnancy, thus preventing superfetation. The few described cases can be explained on the basis of a retardation in development of a less advanced twin. In other mammals, however, 16 probable cases have been reported: King ('13), two cases in albino rats; Sumner ('16), two cases in mice; Smith ('27a, b), 4 cases in sows and 2 cases in sheep; Slonaker ('34), two cases in rats; Markee and Hinsey ('35), one case in the cat; Stowell ('41), one case in the mouse; Littleford and Gysin ('44), two cases in mice. The essential indication for superfetation was the delivery of a second litter at a shorter interval after the first delivery than the normal gestation period. In mice and rats most of the investigators found an interval of 12 to 16 days between the delivery of the two litters, in one case Littleford and Gysin reported 7 days. The normal gestation period is 21 days.

A rapidly succeeding birth of a second litter can be due to a retardation in development of some of the fetuses from the same set of eggs. Weichert ('42) stated that a second pregnancy probably never occurs and that cases of superfetation may be explained by variations in time of implantation among the same set of fertilized eggs. He called this

¹ Aided by a grant to Warren H. Lewis from the American Cancer Society on recommendation of the Committee on Growth and by funds from Dr. Henry Winsor, Philadelphia.

parallel embryonic development. He based this on finding differently advanced fetuses in each uterine horn of a rat.

Superfetation should be defined as ovulation, fertilization, and implantation of a second set of eggs during pregnancy. Oestrus and ovulation during pregnancy have been described by Long and Evans ('22, rat); Nelson ('29, rat); Swezy and Evans ('30, rat) and Watt ('31, mouse). Crew and Mirskaia ('30) and Watt ('31) reported copulation during pregnancy. According to Weichert ('42) and Littleford and Gysin ('44), however, no one has demonstrated the occurrence of both ovulation and copulation (fertilization) in a pregnant animal.

REPORT OF CASE

This case was found in the course of many unsuccessful attempts to obtain cleavage in vitro of fertilized eggs cut out of the uterine tubes. On December 11, 1948 a cage containing two white females and one white male in which previous mating had not been observed was obtained. On December 13, at 3:30 P.M., one female showed a vaginal plug, a sign that mating had occurred. On December 14, at 11 A.M. the mouse was killed. The uterus was swollen and the blood vessels considerably filled. The right horn showed 4 and the left horn three, distinct circumscribed swellings which extended toward the mesometrium (fig. 1). When the uterus was opened, ellipsoid thickenings of the decidua surrounding the implanted eggs were found. A small lumen was located on the antimesometrial side (fig. 3).

By cutting up the uterine tubes 5 eggs were obtained, three from the right and two from the left side. All of them were in the one cell stage and each one had two polar bodies and a perivitelline space. Each egg was mounted in diluted Gey solution on a hollow ground slide and kept in the incubator at 37°C. At 2 P.M. one egg had divided into two cells. This normal cleavage together with the finding of a vaginal plug on the previous day and an open uterine lumen indicate that fertilization of this egg had occurred. The egg did not

divide further and died shortly after the first division. All of the other eggs stayed in the one cell stage until they died, the fate of all one-celled eggs that I attempted to cultivate.

The right ovary showed 4 and the left 3 prominent corpora lutea which were bright pink in contrast to the pale ovary. Two freshly ruptured follicles in each ovary were found to have a red blood point in the center (fig. 2).

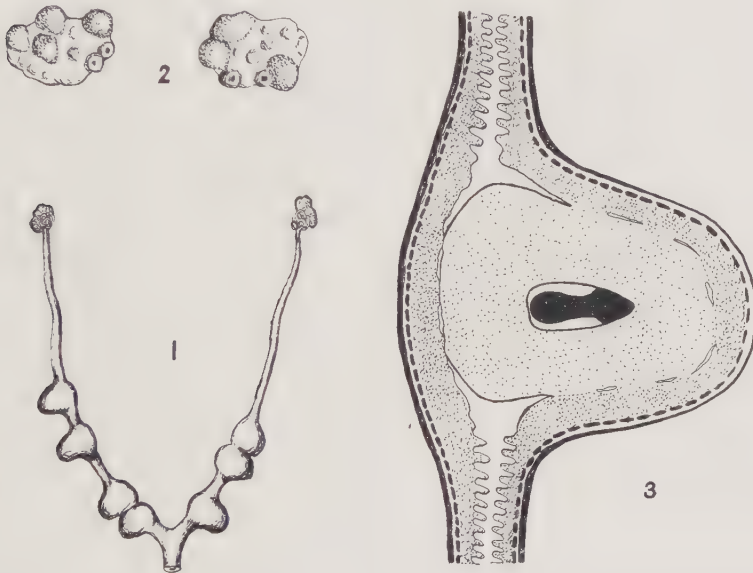


Fig. 1 Ventral view of reproductive tract of the mouse described showing the implantation sites in the uterine horns.

Fig. 2 Right and left ovary. Corpora lutea dotted. Two freshly ruptured follicles in each ovary.

Fig. 3 Longitudinal section through one of the implantation sites. Decidua capsularis surrounding the embryo is considerably swollen. Old uterine lumen dorsal to the embryo (right side of the diagram) is almost closed. New lumen ventral to the embryo (left) reestablishes a straight connection through the uterine cavity by about $8\frac{1}{2}$ days. (After Burekhard, '01, and Snell, '41).

Attempted production of superfetation

Many attempts to produce another case of superfetation met with no success. Thirty-eight females in 4 to 7 days preg-

nancy were caged with males for further 7 to 11 days. Neither mating nor vaginal plugs were observed, and ova in the tubes were lacking.

Uterine lumen

In order to find out if the sperms can reach the oviducts in spite of the implantation bulges, methylene blue was injected into the uteri of mice 8 to 10 days pregnant. In each case the fluid easily passed through the lumen and filled the tubes. In this stage of decidual growth the sperms thus should be able to ascend into the oviduct. This is in accordance with the findings of Burckhard ('01) and Snell ('41). The latter stated: "Further growth of the decidua constricts and finally, by about 8 days, completely closes the uterine lumen dorsal to the embryo except for one or more small isolated chambers. On each side of the decidual swelling the uterine lumen remains open, but at this period in development there is no continuous passage throughout the length of the uterus. A little later a continuous lumen is reestablished, but the new lumen is on the opposite side of the decidual swelling from the old, passing ventral instead of dorsal to the embryo. An early stage in this reestablishment of the lumen may be seen at about 8 days." (See fig. 3.)

Egg migration

In one mouse three implantation bulges were found in the right and 5 in the left horn. The lowest bulge of the left side was located close to the bifurcation 4 mm distant from the next bulge. The other bulges of this side were closer together. Each of the ovaries, however, had 4 corpora lutea. Since eggs cannot escape into the abdominal cavity because of the ovarian capsule, internal migration from the right to the left horn must have occurred in the uterus.

DISCUSSION

This observed superfetation indicates that two separate ovulations and fertilizations occurred; the first 8 days and

the second one day before the animal was killed. According to Burekhard ('01) and Lewis and Wright ('35) eggs do not implant before the 5th day of development. In the present case the decidual swellings were as big as the usual 8 or 9 day ones. We must assume a second ovulation during pregnancy, for both ovaries showed freshly ruptured follicles. At this time a second copulation took place. The observed vaginal plug belongs to this second copulation. Since the eggs were found in the one cell stage, and one egg divided soon afterwards, ovulation occurred about 24 hours before the mouse was killed. (Lewis and Wright, '35, and others determined the average duration of the one cell stage to be 24 hours.) There was thus a fertilization interval of about 8 days between the two sets of eggs, the duration of two oestrus cycles of 4 days each.

In the reported case it does not seem likely that the sperms of the first (not observed) copulation could have fertilized both sets of eggs, as Sumner ('16) assumed. Lewis and Wright ('35) observed only non-motile sperms in the tubes 12 hours after mating.

Both ovaries acted simultaneously, for corpora lutea as well as ruptured follicles were found in both. The eggs of each ovulation had reached the same developmental stage in the right and left sides of the reproductive tract.

In pregnant mice the implantation sites are usually located in the lower half of the uterine horns, close to the vagina. The eggs of a second set, therefore, could implant in the upper half. This order of implantation would eliminate the difficulty in explaining how the older litter could be delivered without disturbing the younger one.

It is not possible to deny that the delivery of two litters within a few days can be due to a retardation in development of the second set or to a parallel embryonic development as described by Weichert. The present observation, however, indicates that real superfetation is possible if a second set of eggs can implant in the upper part of the uterus.

I want to express my sincere thanks to Dr. Warren H. Lewis for having given me the opportunity to carry out these investigations.

SUMMARY

1. A mouse with 7 implanted 8-day ova had a recent vaginal plug, signs of fresh ovulation in both ovaries and one-celled eggs in the tubes. One egg divided in vitro indicating fertilization.

2. The uterine lumen is open at this 8-day stage and implantations usually occupy only the lower halves of the uterine horns. Superfetation is possible, therefore, if such a second set of fertile eggs can implant in the upper part of the uterus.

3. Attempts to produce other cases of superfetation failed.

4. Indication of egg migration from one uterine horn to the other was found in one animal.

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RETARDATION IN ONSET OF OSSIFICATION IN CHIMPANZEE RELATED TO VARIOUS ENVIRONMENTAL AND PHYSIO- LOGICAL FACTORS¹

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Age-norms for onset of ossification in 70 epiphyses and short bones of the left extremities in chimpanzees have been reported by Nissen and Riesen ('49). The 16 animals of this normative group were raised under uniform conditions of nutrition, handling, and environment. Comparable data for 43 young chimpanzees subjected to radical experimental procedures, or raised under deviant environmental conditions, are presented below. Earlier studies on man and other animal forms suggest that some of our variables (e.g., castration) might be reflected in speed of ossification (see, for instance, Talbot, '39; Francis, '40; Sontag and Lipford, '43; Noback, Barnett and Kupperman, '49). We know of no previous work, however, which would indicate that a factor such as light-deprivation during the early months of life (see below) would retard the appearance of ossification centers.

The several variables considered in this paper are as follows:

I. Controls. Animals presumably raised under the same conditions as the 16 original normative animals but born too

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late to be included in that group. These animals may be considered a "control" for any unsuspected long-term changes in nursery conditions; they are approximate contemporaries of the animals in Groups VI, VII, and VIII. ($N=3$)

II. Mother-reared. Eighteen infants² were raised by their own chimpanzee mothers for the first 6 to 22 months of life (average: 11.7 months). Since the chimpanzee mother rarely allows her infant to take any food other than milk from the breast for the first year of life, the nutrition of these youngsters was restricted in variety and probably quantitatively, by comparison with the normative group. Weight-increase during the first year of life is approximately twice as great for the nursery-raised as for the mother-raised infant. These infants also had to use their muscles much more—in holding on to the mother—than did those raised in the nursery cribs. ($N=18$)

III. Home-reared. Three infants were raised, individually, in a human home environment for the first year of life or longer. These animals presumably enjoyed greater variety in regard to food, stimulation, and opportunity for motor expression than did members of the normative group. ($N=3$)

IV. Early deaths. Several animals died within a few days or months after birth; their health-records suggest an innate constitutional inferiority. ($N=7$)

V. Stillbirths. These cases are considered separately from the preceding group since parturitional accidents or anomalies, rather than constitutional defect of the infant, probably were responsible for death. ($N=4$)

VI. Castrate. A fairly complete series of roentgenograms is available for one male infant, castrated at the age of two months. ($N=1$)

² About half of these animals, as well as Alpha of Group III, were born before our examinations at the Laboratories were started. Most of these early roentgenograms were made by Dr. W. McL. Shaw of Riverside Hospital, Jacksonville; some were made at the Yale Medical School. We are grateful for this help and for that of various past members of the Laboratory staff who made these early records possible.

VII. Brain operations. Bilateral extirpation of frontal cortical association areas was undertaken on one male (Alan) at age of two months. In another male (Ked), surgical removal of frontal association areas was done at age two months, of parietal association areas at three months of age. (Precise determination of the location and extent of these lesions will have to await post-mortem examinations.) ($N=2$)

VIII. Light-deprivation. Four animals were kept in complete (Faik, Debi) or near-complete (Snark, Alfalfa) darkness during early periods of life. The age-periods of such light-deprivation were: Snark, birth to 33 months; Alfalfa, birth to 20 months; Debi, birth to 7 months; Faik, 8 months to 24 months. ($N=4$)

IX. Restriction of tactual and grasping experience. One male was raised from birth with heavy cardboard tubes enclosing hands, forearms, feet and lower legs. Although he could walk quadrupedally, he was not able to grasp or manipulate objects (except with his mouth). ($N=1$)

In addition to the foregoing, we present also some fragmentary data on ossification centers present in three fetuses born 115, 158 and 177 days, respectively, after estimated data of conception.

COLLECTION AND TREATMENT OF DATA

Whereas roentgenograms of members of the normative group were made at frequent and regular intervals, permitting precise estimation of the age at which each center began to ossify, the animals here under consideration were necessarily examined less frequently. Only one set of roentgenograms, for instance, was made of each stillborn animal. For the mother-raised group there usually was no information prior to separation. Each of the present determinations of acceleration or retardation is therefore based on less than the total of 70 centers taken into account in the case of the normative animals. Furthermore, intervals between exami-

nations usually being relatively long, interpolation of onset-ages (see Nissen and Riesen, '49) was not attempted.

Most of our data, therefore are presented in terms of "earlier" or "later" than (a) the mean, and (b) the lower and upper limits of the range, of the normative group. Such data do not give a measure of the amount of acceleration or retardation. They do indicate direction of difference, however. Consistency of trend among all individuals comprising a group contributes to the probability that the factor or condition characterizing that group is responsible for the indicated direction of difference. Such probability, of course, is proportional to the extent of the available data (number of individuals and number of dated centers for each).

RESULTS AND DISCUSSION

The data for all 43 subjects, grouped according to the 9 factors or conditions described above, are summarized in table 1. The various indices used are explained in the legend of the table.

TABLE 1

Results for 43 subjects, grouped according to 9 classes of deviant factors or conditions

Column 1: N = number of centers for which relative onset-determinations are available.

Column 2: Max. age = highest age in months at which roentgenograms are available.

Column 3: Range: Percentage of centers appearing before the lower limit of the range (—LL), and after the upper limit of the age-range (+UL) of the normative group.

Column 4: Mean age: Percentage of centers appearing earlier (—) or later (+) than the mean age of the normative group. The percentage of centers appearing exactly at the normative mean age is shown in the (=) column. (The average percentage for each group is a simple average of the percentages of the individuals in that group. Except for group III, the figures in columns 3 and 4 are almost the same when the group average takes into account the differing N's for the several individuals.)

Column 5: Mn Z-score: Z-score computed on basis of means and S.D.'s of the normative group only. This figure reflects both the relative incidence of below- and above-average scores and the magnitude of the differences. The N for these computations is usually considerably less than that shown in column no. 1. The "average Mn Z-score" was obtained by adding all individual Z-scores and then dividing by the N of the group, thus taking into account the differing N's for each individual.

TABLE 1 (continued)

	1	2	3		4			5
	N	MAX. AGE	% OF CASES, RANGE		% OF CASES, MEAN AGE			MN Z-score
			— LL	+ UL	—	=	+	
<i>I. Controls</i>								
Buff	33	24	2	9	49	12	39	— .12
Dehn	29	24	3	3	45	17	38	— .30
Hank	55	24	2	9	24	11	65	+ .30
Average (232) ¹	39	24	2	7	39	13	47	+ .05
<i>II. Mother-reared</i>								
Brinka (22 mos.) ²	42	48	0	89	0	0	100	+ 3.44
Jada (21 mos.) ²	27	24	0	86	4	0	96	+ 3.09
Mu (16 mos.) ²	16	19.5	0	70	6	0	94	+ 1.29
Beta (14 mos.) ²	43	37	0	100	0	0	100	+ 4.53
Bula (14 mos.) ²	17	36	0	13	0	0	100	+ 1.54
Bob (13 mos.) ²	21	17.5	0	46	5	0	95	+ 1.41
Fauna (13 mos.) ²	29	42	0	81	7	0	93	+ 1.57
Helene (13 mos.) ²	11	17	0	18	9	0	91	— .06
Tom (13 mos.) ²	17	17	0	24	0	0	100	+ .91
Ben (12 mos.) ²	10	15	0	0	20	0	80	
Dick (12 mos.) ²	18	17	0	36	0	0	100	+ 1.41
Delta (10 mos.) ²	23	12	0	38	13	0	87	+ .64
Gamma (9 mos.) ²	34	26	3	90	3	0	97	+ 2.74
Becky (7 mos.) ²	22	12	3	56	14	0	86	+ .83
Gua (7 mos.) ²	45	27	0	48	0	0	100	+ 1.80
Mars (6 mos.) ²	64	66	0	59	9	6	85	+ 2.68
Verb (6 mos.) ²	42	24	2	16	17	2	81	+ .65
Frans (3½ mos.) ²	18	24	4	17	33	0	67	+ .78
Average (223) ¹	28	27	1	49	8		92	+ 2.20
<i>III. Home-reared</i>								
Alpha	48	18	19	2	83	0	17	— .92
Fin	17	54	0	79	0	0	100	+ 2.05
Viki	26	12	0	13	31	11	58	+ .46
Average (238) ¹	30	28	6	31	38	4	58	+ .09
<i>IV. Early-deaths</i>								
Bona (7 mos.) ³	20	7	0	23	25	15	60	— .11
Japa (4.5 mos.) ³	3	4.5	0	0	33	0	67	
Gudi (4 mos.) ³	18	3	0	77	17	11	72	— .42
Cub (18 days) ³	8	0	0	75	0	37	63	+ 1.78
Fubsy (12 days) ³	12	0.3	0	40	25	17	58	— .42
Febe (7 days) ³	11	0	0	40	28	18	54	— .42
Balfa (3 days) ³	11	0	0	0	36	28	36	— .44
Average (223) ¹	12	2	0	36	23	18	59	— .22
<i>V. Stillbirths</i>								
Vekar	8	0	0	0	25	63	12	— .44
Stib	9	0	0	80	11	33	56	— .64
Hej	6	0	0	0	40	50	10	— .52
Infant (Nana's)	8	0	0	0	25	63	12	— .44
Average (220) ¹	8	0	0	20	25	52	23	— .50
<i>VI. Castrate</i>								
Dag (213) ¹	60	48	0	56	7	0	93	+ 2.12
<i>VII. Brain operations</i>								
Alan	31	12	16	0	68	16	16	— .87
Ked	18	12	8	9	61	0	39	— .89
Average (221) ¹	25	12	12	5	65	8	28	— .88
<i>VIII. Light-deprivation</i>								
Alfalfa	33	48	0	26	27	0	73	+ .56
Debi	23	12	3	61	4	0	96	+ 1.23
Faik	38	24	0	72	0	0	100	+ 2.90
Snark	56	48	2	18	12	9	79	+ .83
Average (217) ¹	38	33	1	44	11	2	87	+ 1.20
<i>IX. Restriction of tactual and grasping experience</i>								
Rob (212) ¹	27	12	0	78	4	0	96	+ 2.17

¹ Average estimated gestation period for group, in days.² Age at separation from mother.³ Age at death. See text for explanation of the many negative Mn Z-scores in this group.

Retardation is indicated when the second (+ UL) figure in column 3 is greater than the first (— LL) figure. This means that a higher percentage of the centers (for which we have data) appeared later than the upper limit of the range of the normative group than appeared before the lower limit of the normative range. (If our normative group had been larger, these percentages presumably would be smaller. In general, high percentages in *both* (— LL) and (+ UL) indicates greater *variability* than that of the normative group.)

Similarly, a higher figure in the plus (+) than in the minus (—) column of 4 indicates retardation; more centers appeared at an age above the normative mean than first ossified at an age below the normative mean. Contrariwise, a preponderance of minus over plus percentages, in either 3 or 4, indicates acceleration.

In column 5 is shown the "Mean Z-score." For each center (only those centers for which an exact onset-age was available could be used here) the deviation of its onset-age from the normative mean onset-age was divided by the S.D. of the normative group. All such Z-scores were then averaged, algebraically. (See also legend of table 1.) Since the number of centers available for such treatment is usually small, less emphasis is placed on this index than on the relative percentages of columns 4 and 5. Again, a minus figure indicates acceleration, a plus figure retardation.

In connection with the following discussion of each group, the reader may wish to refer back to the descriptions of the several variables which were presented above.

I. Controls. The fact that the difference between the percentages of centers in this group which appeared before and after the normative mean is small (8%), and that the three mean Z-scores are all relatively low, averaging only 0.05, suggests that there were no unsuspected long-range changes in nursery conditions which might have affected rate of ossification. The normative-group scores are, therefore, acceptable as criteria for indicating accelerating or retarding effects of the special factors considered in this paper. On the other

hand, these figures give some notion of the order of magnitude which must be expected in columns 3, 4, and 5 to indicate the operation of an influence on ossification-rate.

II. Mother-reared. According to all percentage criteria of columns 3 and 4, the 18 mother-reared animals are considerably retarded by comparison with the normative group. The mean Z-scores all show the same trend, with one exception: it happens that we have only two precise datings for Helene, and of these the minus Z-score is a little larger than the plus score. It will be noted that the animals have been listed in the order of decreasing length of time spent with the mother; there is some suggestion that degree of retardation decreases as this period decreases.

III. Home-reared. One of these animals (Alpha) is definitely accelerated; the other two show retardation. From information available, it seems probable that Alpha was given all the food she would eat, whereas some effort was made to keep Viki from gaining weight too rapidly. Alpha has been perhaps the healthiest (as well as one of the largest) animals in the colony (she is now 19 years old). Fin had several periods of acute illness, as well as a hernia operation, during infancy.

IV. Early deaths. In general it seems fair to characterize these animals as constitutional inferiors. Bona died, at age 7 months, of undiscovered causes; she had been "sickly" during much of her short life. Japa died of dysentery about a month after unilateral extirpation of cortical areas 4 and 6. Gudi suffered from a series of infections (abscesses) which were treated with sulfa drugs and penicillin. She died of a terminal pneumonia. Cub had a severe diarrhea (presumably a "virus dysentery") starting soon after birth; his treatment was directed by a competent pediatrician, but he died after 18 days of constant nursing care. Fubsy, Febe, and Balfa died of early pneumonias. Eleven of the 14 percentage indices (columns 3 and 4) indicate retardation, none show acceleration. The preponderance of negative mean Z-scores is not significant; it happens that for this group there were

relatively few exact (absolute) age-determinations for centers which appeared late.

V. Stillbirths. These animals were born after gestational periods within the range of the normative group (233, 224, 217 and 204 days, respectively). Their birth weights and general external development showed no abnormalities. Vekar probably was born alive (since autopsy showed air in the lungs) but when first seen by us his mother, Vera, had consumed part of the infant's body. (Placentophagia is common among chimpanzees — see Nissen and Yerkes, '43 — but this is the only case here on record of a mother eating her young.) We assume that the prenatal period in these animals was normal and that death was the result of injury during birth or immediately thereafter. As may be seen in table 1, as a group they had about the usual number of ossification centers at birth.

VI. Castrate. The roentgenographic record of Dag is quite extensive; complete sets of pictures were taken at 6, 8, 10, 12, 15, 18, 21, 24, 30, 36, and 48 months, giving onset-data for 60 centers. The statistics are consistent in showing marked retardation.

VII. Brain operations. Both Alan and, to a lesser extent Ked, show some acceleration. It obviously would be premature to suggest an explanation for this apparent relationship.

VIII. Light-deprivation. All 4 subjects show marked and consistent retardation. The reason for this effect is obscure. The food-intake of these animals did not deviate noticeably from that of the normative group. Records of "spontaneous activity" (Snark and Alfalfa only) indicate that although the diurnal distribution of their activity differed from that of the normative group, the total amount of time spent in gross bodily movement was essentially the same as for the normals. A relationship between light and glandular function, such as that found in birds (e.g., Rowan, '38), might be considered.

IX. *Tactual-manual restriction.* Rob shows marked and consistent retardation. Detailed analysis of the data indicates that this retardation is *general*; it is no more pronounced in the epiphyses of the phalanges, for instance, than in those of the arms and legs or in the short bones of wrist and ankle. It may be remarked that gross bodily activity of this subject was not noticeably less than that of other animals, and that at age 15 months no signs of muscular atrophy or underdevelopment could be observed in any region of the body.

It may be noted that with the possible exception of group VII, none of the factors or conditions here under consideration produced, on the average, an *acceleration* in onset of ossification. Apparently the regime of nutrition and care obtaining in the nursery where our normative group was raised, approaches the optimal in this respect. A survey of the findings as a whole suggest 5 causes of retardation in ossification onset: (a) quantitative and/or qualitative nutritional insufficiencies, (b) constitutional inferiority, (c) castration, (d) certain deficiencies of stimulation, and perhaps the limitations of specific behavior associated therewith. (Amount of general activity does not seem to be a critical factor.)

The 5th factor (e) contributing to retardation was considered in our report on the normative group (Nissen and Riesen, '49): "Extremely short gestation periods are accompanied by retardation (as measured from birth) in average age at appearance of the centers; moderate variations in gestation length appear to be unrelated to mean ossification age." In the present list of 43 animals there are at least 5 whose gestations were "extremely" short: Faik (196 days), Nana's stillborn infant (204 days), Tom (206 days), Helene (206 days), and Cub (207 days). In table 1 are shown the mean gestation ages for each group; many of these, it will be noted, are shorter than the normative group average of 226 days. This fact indicates the necessity for caution in attributing retardation to the other factors above-mentioned — especially when a "group" consists of just one case (VI

and IX). The case of Rob (IX) is especially in point: not only did he have a short gestation (212 days), but he was greatly retarded in ossification at birth (i.e., before the special restriction was imposed). Only the calcaneus and talus started ossifying prenatally in Rob, whereas in the median normative male 8 other centers are already visible at birth.

Data on fetuses

If we include the calcaneus and talus (present before birth in all normative subjects), the median number of centers in which onset of ossification began prenatally in the normative group is 10 for males, 12 for females. For the three fetuses the corresponding figures are one, three, and one, respectively. In two of the fetuses, identified as males, born 115 days and 177 days after estimated date of conception, only the calcaneus had begun to ossify. In one fetus, whose sex was not determined, having an estimated conception-age of 158 days, ossification had begun in the calcaneus and in the epiphyses of the head of the humerus and head of the femur (but not in the talus).

SUMMARY

Retardation in the onset of ossification of epiphyses and short bones of the extremities (by comparison with a "normative" group of chimpanzees raised under standardized nutrition and care) was found to be associated with the following deviant conditions: (1) rearing of the infant by its own mother in captivity ($N=18$); (2) general constitutional inferiority ($N=7$); (3) early castration in the male ($N=1$); (4) light-deprivation for a long period during infancy ($N=4$); (5) restriction of tactual experience and opportunity to grasp ($N=1$). Nutritional inadequacy, constitutional inferiority, castration, and deficit of sensory stimulation, appear to be the main factors contributing to such retardation. However, the small number of cases in some of our groups, and the concomitant variations in length

of gestation, indicate that these interpretations must be considered tentative and suggestive, rather than conclusive.

Certain brain operations (ablation of cortical association areas) early in life, had no marked effect on age of ossification-onset ($N=2$). Animals dying as the result of parturitional accidents after apparently normal gestation show no retardation in prenatal appearance of ossification centers ($N=4$).

Fragmentary data on ossification centers present in three chimpanzee fetuses are presented.

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THE COMPARATIVE DISTRIBUTION OF ESTERASE IN THE TISSUES OF FIVE MAMMALS BY A HISTOCHEMICAL TECHNIQUE¹

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SIXTEEN FIGURES

In a previous communication, a new histochemical method for the demonstration of non-specific esterase was described by Nachlas and Seligman ('49a). This consisted in incubating sections of tissue, which had been fixed in acetone and embedded in paraffin, in a buffered solution at pH 7.8, containing the substrate, beta naphthyl acetate, and a stabilized salt of diazotized alpha naphthylamine. At the sites of enzymatic activity, the substrate was hydrolyzed to beta naphthol which immediately coupled with the diazotized naphthylamine to form a purplish-red, insoluble, azo dye (see color plate of previous paper by Nachlas and Seligman, '49a).

A preliminary exploration of the distribution of the enzyme thus demonstrated revealed a distribution similar to that found by Gomori ('46) with a histochemical method for lipase, using "Tween"³ as a substrate. Evidence that both methods demonstrate non-specific esterase has been provided recently by Huggins and Moulton ('48); Nachlas and Seligman ('49a,

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² Research fellow of the National Cancer Institute.

³ Stearic acid ester of polyglycol and sorbitan.

b); Seligman, Nachlas, and Mollomo ('49); Seligman and Nachlas ('50) and Seligman, Nachlas, Manheimer, Friedman, and Wolf ('49). A comparison of the distribution of esterase in the tissues of man, dog, rabbit, guinea pig and rat forms the subject of this paper.

MATERIAL AND METHODS

Tissue was obtained from freshly killed animals, human autopsy material and surgical specimens. Sections, 6 μ thick, were prepared from the tissues, fixed in acetone and embedded in paraffin according to the procedure previously described. The preparation of the substrate, beta naphthyl acetate, and the stabilized diazonium salt, together with the procedure for staining and mounting the sections, are described elsewhere by Nachlas and Seligman ('49a). In some sections counter-staining with hematoxylin was done. All photomicrographs were taken through a green filter.

RESULTS

Tissues were examined from 10 rats, 4 rabbits, 4 guinea pigs, 6 dogs and 6 human subjects. Esterase was abundant in many tissues. Tissues known or demonstrated to contain esterase never failed to stain by this method. Areas which did not show esterase might have done so had a longer period of incubation than 20 minutes in a fresh solution of substrate been used. Although there was frequent variation in intensity of staining, the pattern of distribution obtained in any given tissue of a single species was generally constant. Among different species, however, the pattern of distribution and activity of the enzyme varied considerably for similar tissues. Esterase was found in both cytoplasm and nuclei, although generally stronger in the former, and not infrequently absent in the latter. The distribution in the cytoplasm was usually uniform except in certain cells where it was localized in the supranuclear region (see intestinal glands).

DIGESTIVE SYSTEM

Esophagus. Esterase activity was limited to the mucosa. Staining was most intense in the epithelium, increasing toward the stratum germinativum. Both the lamina propria and the muscularis of the rat's mucosa were weakly positive for esterase. In the dog, the esophageal glands and muscularis mucosae were lightly stained. No esterase activity was seen in man.

Stomach. Esterase activity was found in the mucosa of the cardia and body in all species. None was seen in the antrum or pylorus. However, there were numerous differences in both localization and intensity among the animals studied (figs. 1-4). Most of the glands in the rabbit's mucosa were strongly positive for esterase, and all of the muscular layers contained the enzyme in lesser amount (fig. 1). In the dog, the staining was prominent in the surface epithelium with a rapid decrease in activity below the neck cells (fig. 2). All muscular layers contained a small concentration of enzyme. The guinea pig stomach resembled that of man (fig. 3). The greatest activity was at the base of the mucosa, less in the lamina propria mucosae, and none was noted in the muscular layers. Some sections of human stomach were intensely stained (fig. 4) and others were weakly stained. Dye was concentrated in the supranuclear region of the cells. The cytoplasm of the parietal cells of all species were more intensely stained than the zymogenic cells. This was particularly striking in the rabbit and in man (figs. 1 and 4). The nuclei of these darkly stained cells were pale or colorless. The nerve cells and surrounding connective tissue of Auerbach's and occasionally of Meissner's plexus contained esterase, most marked in the dog. The small arteries and veins in the submucosa were occasionally stained.

In the antral and pyloric regions which generally showed little or no esterase activity, there was faint and irregular mucosal staining in the rat, guinea pig, and dog. The nerve plexuses in these areas, when seen, contained esterase.

Small intestine. In the rat, rabbit, and guinea pig, the surface epithelium of the duodenal villi stained prominently, whereas the connective tissue of the villi and Brunner's glands stained faintly and irregularly. In the dog, very little of the surface epithelium was stained but the lamina propria in the villi, the sub-mucosal arteries and the nerve plexuses showed esterase. All muscular layers were heavily stained in contrast to other species which showed little or no esterase activity in the muscular coats. In man, Brunner's glands were weakly positive for esterase.

Species differences were shown by the jejunum. The surface epithelium of the rat's villi and the lamina propria of that region stained strongly with a diminution toward the base of the crypts (fig. 5). Some areas clearly demonstrated that esterase activity was concentrated in the supranuclear portion of the cells. The circular muscle and occasional sub-mucosal blood vessels were weakly positive for esterase. In the guinea pig, only the cells bordering the ducts of the glands were stained slightly. Esterase activity in the dog was noted in the villous portion of the lamina propria mucosae in what appeared to be smooth muscle cells (fig. 6). Esterase was also present in blood vessels, Auerbach's plexus, the muscularis mucosae, the inner fibers of the circular muscle, and the entire longitudinal muscular layer. No esterase activity was found in the jejunum of man.

A similar pattern of distribution was seen in the ileum, except that the enzyme activity was less and sometimes irregular.

Large intestine. The appendix of the dog contained esterase prominently in the muscularis mucosae, longitudinal muscular layer and plexus of Auerbach. The mucosa was essentially free of enzyme.

The colonic mucosa of all species contained esterase. In the rat, it was found irregularly in the mucosal glands, and to a lesser extent in the lamina propria. Only the surface epithelium of the colon of the rabbit contained esterase, where it was located in the supranuclear region of the cytoplasm. The cell nuclei did not stain. In both man and dog, esterase ac-

tivity was feeble in the mucosal glands. In the dog the muscularis mucosae, longitudinal muscular layer, and nerve plexuses were relatively rich in esterase. Some of the connective tissue cells in the submucosa contained esterase.

Liver. The cytoplasm of the hepatic cells was intensely stained in all species. Most of the lobules were uniformly red, although occasionally some areas appeared darker than others. This was characteristic of the rat and of the rabbit in the region of the central veins. The Kupfer cells were stained weakly in most of the animals. Generally, the connective tissue did not contain enzyme. The blood vessels were usually positive for esterase. The bile duct epithelium contained a little enzyme (absent in man) and the connective tissue coat was positive in the rabbit.

Gall bladder. The epithelium of the rabbit and guinea pig contained esterase, whereas very little was noted in that of the dog. The lamina propria was weakly stained. In the rabbit, esterase was seen in scattered cells which resembled macrophages. The muscular bundles of the dog stained more strongly than those of other animals. No esterase was seen in the human gall bladder.

Pancreas. Esterase activity was uniformly and intensely demonstrated in the acini of all species. In some areas a supranuclear localization of the enzyme was noted. Slight activity was seen in the pancreatic ducts, and none was noted in the interlobular connective tissue. In man, however, both connective tissue and ducts were rich in esterase. Only in man and dog was weak esterase activity seen in the islets of Langerhans.

URINARY SYSTEM

Kidney. The cortex of the rat's kidney was regularly and intensely stained (fig. 7). The glomeruli were weakly stained. The enzyme was present in all coats of small blood vessels. In the rabbit, more irregularity was noted in the staining of convoluted tubules. The medullary rays were well stained. This was even more striking in the dog (fig. 8), and Henle's

limbs were more intensely stained than some of the convoluted tubules. Cell nuclei and glomeruli were free of esterase. Blood vessels were weakly stained. In both guinea pig and man, the pattern was variable and the intensity of staining was less (figs. 9 and 10). The convoluted tubules and Henle's limbs of some of the nephrons were without esterase. The smaller collecting ducts were stained irregularly. Occasionally blood vessels were weakly stained.

Ureter. The epithelium of the rabbit and dog were stained. In the dog the muscular layers were also stained. Esterase was not seen in the human ureter.

Bladder. The epithelium was stained in the dog, irregularly in the rat, and not at all in the guinea pig and man. The muscularis contained esterase uniformly in the dog and irregularly in the rat. The cytoplasm of nerve plexuses were richly reactive.

Female genital system

Uterus. The endometrium and myometrium were weakly and irregularly stained in the rat, and not at all in the guinea pig. In the dog, the endometrial glands were irregularly but intensely stained, and the myometrium was regularly stained (fig. 16). In women, the endometrial glands were weakly stained, and the myometrium was negative.

Fallopian tube. The epithelium of the rat's salpinx was markedly active for esterase, particularly in the supranuclear region of the cells. In the dog, the glands which were located deep in the mucosa were intensely stained, whereas the epithelium was inactive. The muscular layers were rich in esterase. Staining was not observed in the salpinx of women.

Ovary. The blood vessels and the fat surrounding the rat's ovary were strongly stained, whereas the ovary was weakly stained except for occasional areas of increased activity in some blood vessels, follicles and macrophages. The

dog's ovary was also weakly stained. The guinea pig and human ovaries were free of esterase.

Male genital system

Prostate. The esterase activity of the prostate of rat, dog, and man was variable. The epithelium of glands and ducts was intensely stained in some dogs and not at all in others. In man, slight esterase was seen in some of the prostatic concretions of one prostate, and in another prostate significant staining was observed in the glandular epithelium and in the adjacent connective tissue.

Testis and epididymis. The seminiferous tubules and interstitial cells of the rat were rich in esterase (fig. 11). The epithelium of the epididymis also contained esterase. In one guinea pig, only the epididymis stained weakly for esterase. In the dog, a strong reaction was seen in the tubules with apparently more activity in the spermatogonia than in the more mature forms (fig. 12). The interstitial cells were occasionally well stained. Esterase was not demonstrated in human testis.

Other endocrine glands

Adrenal. There were marked species differences in the esterase pattern of the adrenal. The rat adrenal stained weakly and irregularly in the glomerulosa, the fasciculata, and occasionally in the reticularis. The periadrenal fat and blood vessels were intensely stained (fig. 13). In the guinea pig rare staining was observed in the zona reticularis (fig. 14). Weak staining was observed in the glomerulosa of the rabbit. In the dog, the entire cortex contained esterase, particularly in the glomerulosa and reticularis (see published illustration by Nachlas and Seligman, '49a). Periadrenal fat was not stained. In man, staining was uniformly observed in the reticularis, occasionally extending into the fasciculata in finger-like projections (fig. 15). The adrenal medulla did not contain esterase.

Thyroid. Most of the thyroids did not contain esterase. The colloid of several species stained irregularly and weakly. The strongest reactions were demonstrated in the rat, where both epithelium of the follicle and the colloid were positive. In man, the epithelium of certain follicles were faintly stained.

The thymus of the rabbit and guinea pig contained no esterase. The pituitary of the rabbit and man was unreactive for esterase. Human parathyroid was free of esterase.

Respiratory system

Lung. The bronchial epithelium of the rat revealed marked esterase activity, which was concentrated in the supranuclear regions of the cells (see published illustration [5]). The smooth muscle of the bronchioles, blood vessels, and the septal cells were also strongly positive. The rabbit and guinea pig showed a similar reaction in the bronchial epithelium, but the septal cells were weakly and irregularly stained. The dog revealed marked activity in the smooth muscle of the bronchi and a weaker and variable reaction in both the bronchial epithelium and the septal cells. In man, bronchial epithelium stained weakly and irregularly. Stained macrophages were seen. The remainder of the lung tissue did not contain esterase.

Cardiovascular, lymphatic and hemopoietic systems

Heart. Myocardium contained little or no esterase. In the rat, a faint reaction was noted irregularly throughout the myocardium and particularly near the endocardium. In both rat and rabbit, cells which stained were seen scattered throughout the heart. They resembled nerve cells and may have been part of the conduction system. Human myocardium was free of esterase.

The *spleen* of the guinea pig and man was free of esterase, whereas a faint reaction was present in the connective tissue of the septa of the rat and rabbit. Rabbit and dog contained weak esterase activity throughout the red pulp. In the dog, occasional macrophages were intensely stained.

Lymph nodes of all species failed to stain for esterase. Human bone marrow did not contain esterase.

The staining of arteries and veins was quite variable in different tissues. Where staining was observed, it was confined to the media and adventitia. In certain areas, as for example in stomach of rabbits and dogs, the tunica elastica was intensely stained. Intima was never observed to be stained.

Nervous system. Esterase was not present in cerebrum and cerebellum of the rat, rabbit, guinea pig or man. The spinal cord of dog, rabbit and man was free of esterase. Abundant esterase was seen in the nerve plexuses of the small intestine and bladder of the dog. In the rat, only the cytoplasm of the coeliac ganglion contained esterase. Human peripheral nerve was free of esterase.

Human abdominal *skin* and skeletal *muscle* was free of esterase.

DISCUSSION

Three classes of carboxylic acid esterases are recognized at present. These are cholinesterase, lipase, and non-specific esterase. The first is classified on the basis of predilection for substrates containing choline, the second is classified on the basis of predilection for esters of long chain fatty acids and stimulation by taurocholate, and the last by a predilection for esters of short chain fatty acids and inhibition by taurocholate (Willstatter and Memmen ['24]; Nachlas and Seligman ['49b]; Seligman, Nachlas, and Mollomo ['49]; Seligman and Nachlas ['50]; and Seligman, Nachlas, Manheimer, Friedman and Wolf ['49]). There is considerable overlapping of the actions of these enzymes on various substrates, and substrates which are considered ideal for one enzyme may be hydrolyzed to a certain degree by another. Thus olive oil may be hydrolyzed to a slight extent by esterase (Seligman and Nachlas, '50), and naphthyl acetate may be hydrolyzed to a slight extent by lipase (Seligman, Nachlas, and Mollomo, '49). That acetyl cholinesterase does not hydrolyze naphthyl acetate is clear from the absence of staining in brain.

Huggins and Moulton ('48) observed that paranitrophenyl propionate was hydrolyzed by the same tissues which hydrolyze "Tween." They suggested that the hydrolysis of "Tween" is not specific for lipase. Since the distribution of esterase as demonstrated by naphthyl acetate is essentially similar to the distribution with Tween (Gomori, '46), and since an abundant quantity of lipase is limited to pancreas (Cherry and Crandall, '31; Nachlas and Seligman, '49b; Seligman, Nachlas, and Mollomo, '49; and Seligman and Nachlas, '50), it is probable that the histochemical method of Gomori ('45) for lipase demonstrates both esterase and lipase. If this is indeed the case, then failure to demonstrate esterolytic activity in the stomach of the rabbit, rat, and guinea pig; the intestinal villi and colon of the dog; the kidney and prostate of man with Tween (Gomori, '46), whereas these sites contained esterase according to the hydrolysis of naphthyl acetate, raises the possibility that more than one type of non-specific esterase exists.

The widespread distribution of esterase throughout the tissues of the body and the particular sites where activity was observed to be greatest give us no clue to the function this enzyme serves in the body. Nor is it necessary to assume that the esterase so abundant in cells of liver and pancreas, in epithelium of gut, in the tubules of the kidney, in smooth muscle of arteries, in certain endocrine glands and in nerve plexuses of the gut serves the same metabolic function in each.

Acknowledgement is due Miss Marie Mollomo for technical assistance and Mr. Leo Goodman for the photomicrography.

SUMMARY

The histochemical distribution of non-specific esterase in the tissues of 5 mammals is compared. The tissues of the rat were richest in esterase, those of man were poorest. Liver, pancreas, kidney, lung and gut contained abundant quantities

of esterase in all species. Thyroid was stained only in the rat and adrenal cortex was stained best in the dog.

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PLATE 1

EXPLANATION OF FIGURES

1 Rabbit stomach. Esterase activity is widespread. The parietal cells and layer of circular muscle are stained most intensely. The cell nuclei are free of esterase. An artery in the submucosa contains esterase. $\times 170$.

2 Dog stomach. The surface epithelium and nearby glands contain the most esterase. The deeper zymogenic cells are not stained. The muscular layers are weakly positive. $\times 170$.

3 Guinea pig stomach. Most of the zymogenic cells contain esterase and the parietal cells are even more intensely stained. The cell nuclei do not contain esterase. The muscular layers (center) are weakly positive. $\times 170$.

4 Human stomach. Most of the gastric glands have intense esterase activity. The surface epithelium (top) and muscular layers (bottom) do not contain the enzyme. $\times 170$.

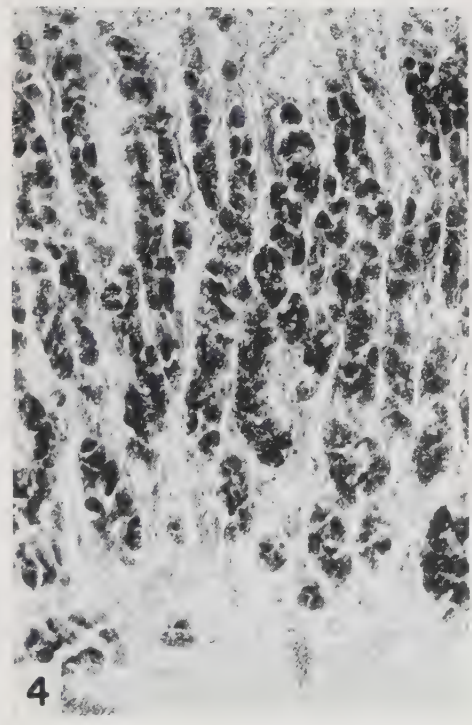
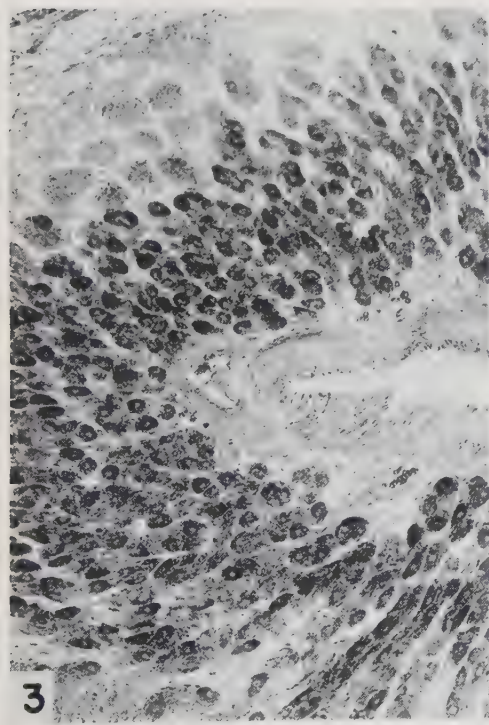
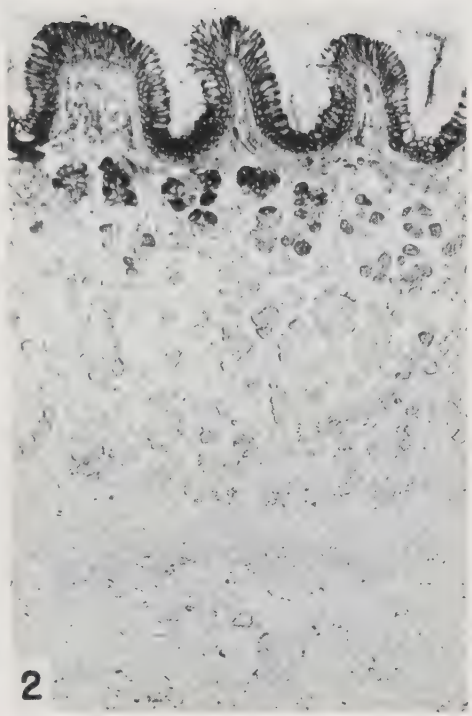
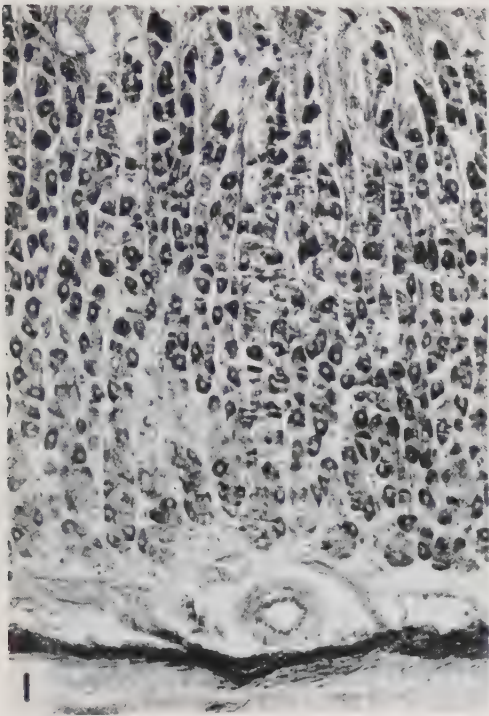


PLATE 2

EXPLANATION OF FIGURES

5 Rat jejunum. The greatest activity is seen in the surface epithelium, less activity is present in the glands of Lieberkuhn and stroma of the villi. The muscular coats (not shown) were weakly positive. $\times 170$.

6 Dog jejunum. The stroma of the villi and particularly what appear to be smooth muscle cells contain esterase. The muscularis mucosae, part of the circular muscle, the longitudinal muscle and Meisner's plexus (not shown) contain esterase. $\times 170$.

7 Rat kidney. The cortex is one of the tissues richest in esterase. The convoluted tubules, Henle's limbs, and blood vessels contain large amounts of esterase. The glomeruli stain weakly. Lightly counterstained with hematoxylin. $\times 170$.

8 Dog kidney. The cortex is rich in esterase. Henle's limbs stain more intensely than some of the convoluted tubules. Cell nuclei are generally free of esterase. Lightly counterstained with hematoxylin. $\times 170$.

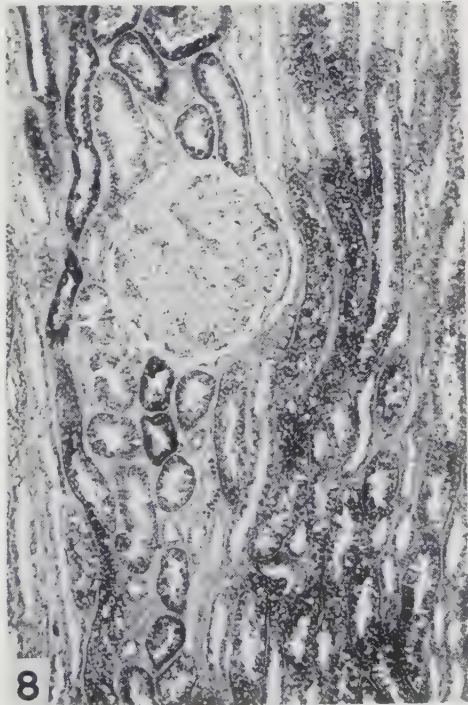
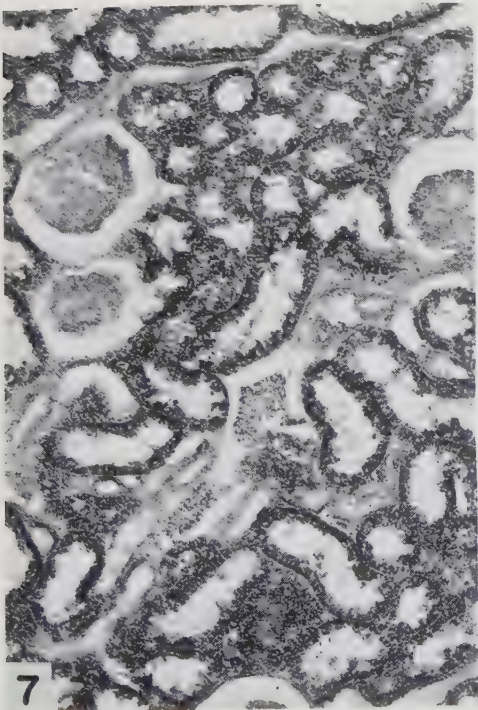
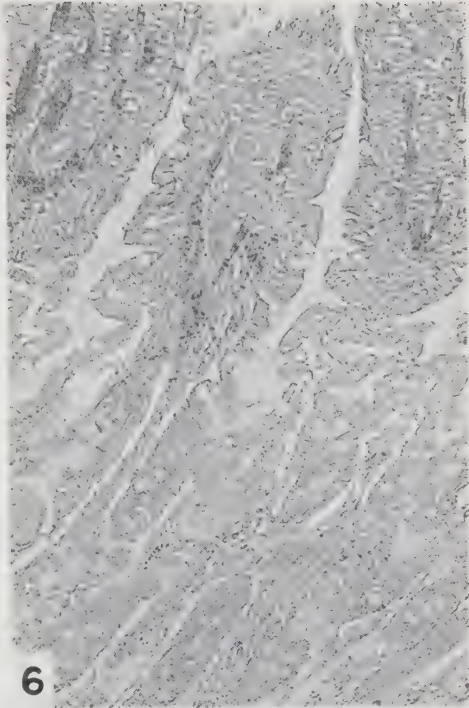
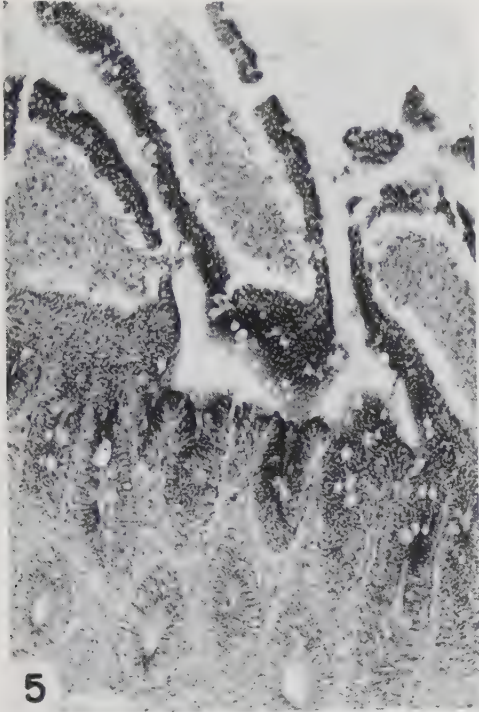


PLATE 3

EXPLANATION OF FIGURES

9 Guinea pig kidney. The esterase activity is low in Henle's limbs and in many of the convoluted tubules. The glomeruli and some of the convoluted tubules are free of esterase giving a patchy appearance to the sections. Lightly counterstained with hematoxylin. $\times 170$.

10 Human kidney. Variation in degree of staining of many of the glomeruli, convoluted tubules, Henle's limbs, and collecting tubules result in a patchy appearance to the section. Lightly counterstained with hematoxylin. $\times 170$.

11 Rat testis. High esterase activity is present throughout the testis. The seminiferous tubules are prominently stained and the interstitial cells are even more intensely stained. $\times 170$.

12 Dog testis. The tubules show less esterase activity than those of the rat. There is variation in the intensity of staining of the tubules. The interstitial cells are occasionally as active as the tubules, but usually are weaker. $\times 170$.

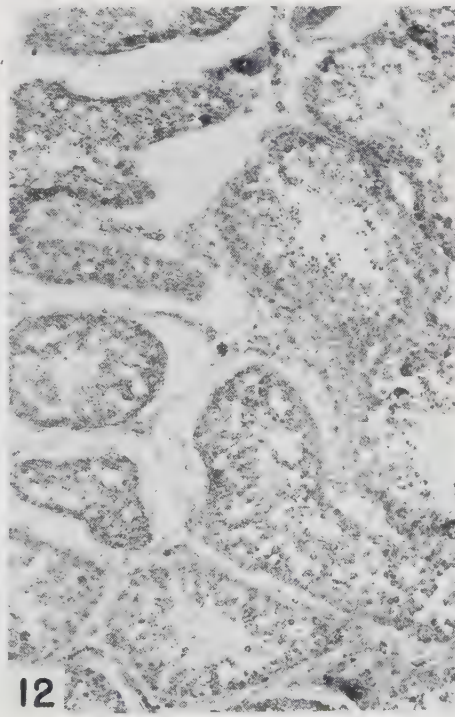
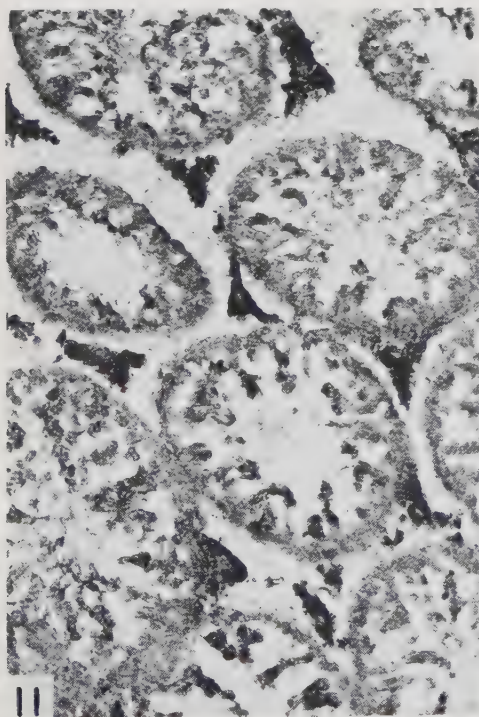
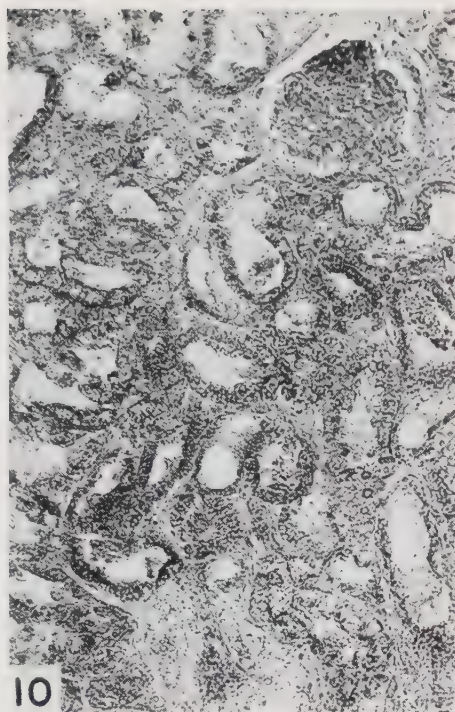


PLATE 4

EXPLANATION OF FIGURES

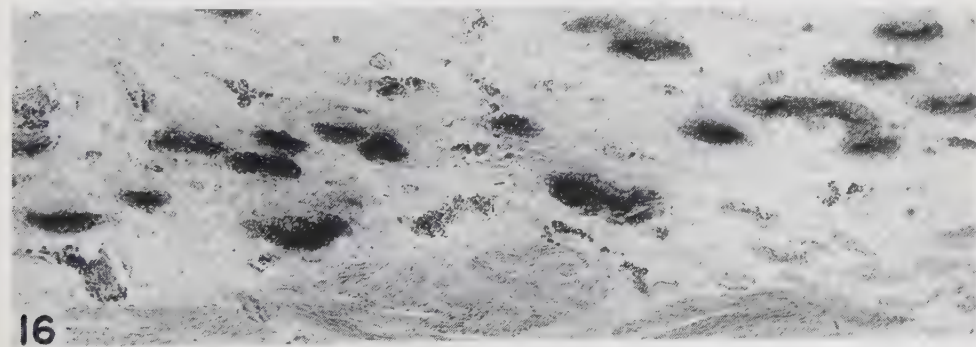
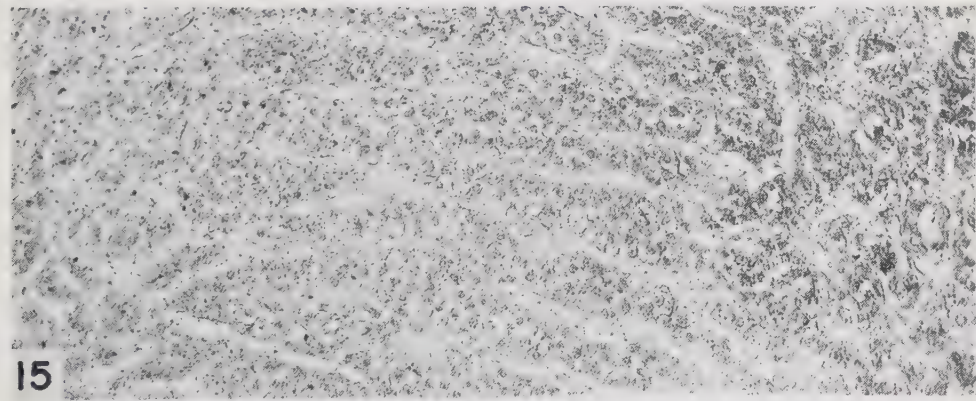
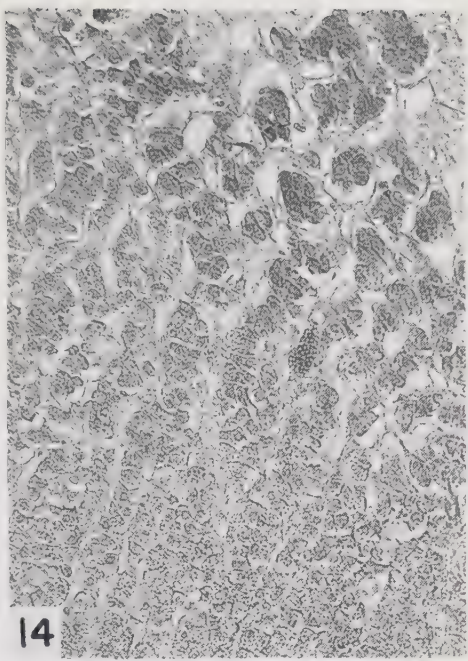
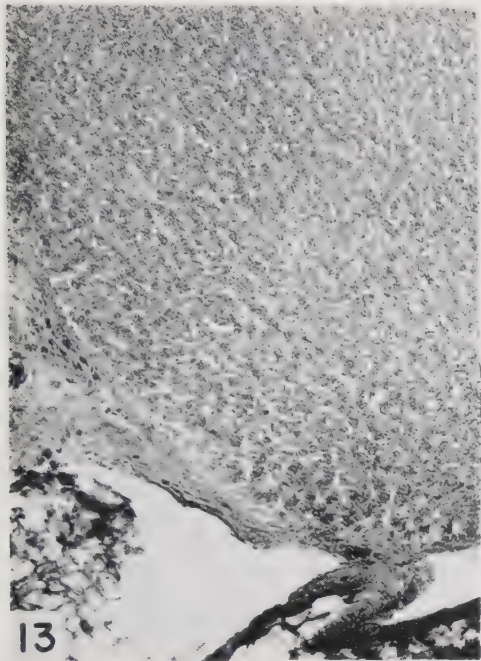
13 Rat adrenal gland. Low esterase activity is evident throughout the cortex. The periadrenal fat and connective tissue cells of the capsule are intensely stained. The medulla (not shown) contains no esterase. $\times 170$.

14 Guinea pig adrenal gland. Groups of cells in the zona reticularis contains variable amounts of esterase. The other zones are free of esterase. $\times 170$.

15 Human adrenal gland. A uniformly positive reaction is observed throughout the zona reticularis and occasional stained cells are arranged in finger-like projections into the zona fasciculata. The other areas do not contain esterase. Counterstained with hematoxylin (some precipitation of hemotoxylin is present). $\times 170$.

16 Dog uterus. The endometrial glands contain abundant quantities of the enzyme, whereas the stroma is unreactive. The myometrium stains regularly for esterase. $\times 170$.

MARVIN M. NACHLAS AND ARNOLD M. SELIGMAN



THE HISTOLOGY OF THE DOG'S LUNG FOLLOWING EXPERIMENTAL COLLAPSE¹

WITH SPECIAL REFERENCE TO THE NATURE OF
THE ALVEOLAR LINING

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TEN FIGURES

INTRODUCTION

This study was undertaken in an effort to learn more concerning the histological structure of the alveolar walls in the mammalian lung, particularly the nature of the alveolar covering. One of us (Loosli, '35) investigated the histological features in the rabbit's lung following collapse by phrenicotomy and pneumothorax. A continuous cellular membrane of endodermal origin lining the collapsed air spaces of the rabbit was not demonstrated, but observations were made only for 40 days after atelectasis was achieved. The technique developed by Adams and Livingstone ('30, '31, '32) and reported in detail by Adams ('33) for producing obstructive atelectasis provided an opportunity to observe the histological changes which might occur following complete collapse of the lung of the dog over a much longer period of time.

MATERIALS AND METHODS

Thirteen apparently healthy young adult dogs weighing from 10 to 14 kg were employed. Bronchial obstruction was

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produced by painting the left mainstem bronchus with 35% silver nitrate solution which resulted, after two to 6 weeks, in complete stenosis and absorption of the residual air in the lung (Adams and Livingston, '30, '31, '32; Adams, '33). Briefly, the technique was as follows: A dog was given morphine (0.015 gm) and atrophine (0.0004 gm) per kilogram of body weight intramuscularly one-half to one hour before bronchoscopy. The animal was then placed on a board in a dorsal position and a bronchoscope introduced through the larynx into the trachea to the level of the carina.

A steel wire (no. 8 gauge, Brown and Sharp) applicator with a firmly secured absorbent cotton tip was dipped into 35% silver nitrate solution. The excess amount of the solution then was wrung from the applicator by pressing the cotton tip to the side of the container. This was done to prevent the cauterizing agent from running into the air passages when the swab was applied to the bronchial mucosa. The swab was then introduced into the left main stem bronchus through the bronchoscope to just above the level of the left upper lobe bronchus. When the bronchus was small the swab was simply thrust into it and held in place for 10 seconds. In larger bronchi the swab was rolled around the entire circumference of the lumen. The white, cauterized area, about 1 cm in width and encircling the entire bronchus stood out in sharp contrast to the normal pink colored mucosa. Occasionally, a second application of silver nitrate solution was necessary before stenosis of the bronchus was complete.

The development of atelectasis of the obstructed lungs was observed by repeated fluoroscopic and x-ray examination of the chest. In this study stenosis was followed by complete absorption of residual air in approximately two weeks after silver nitrate was applied. One dog (no. 17) killed at 16 months was subjected to a left lower lobectomy 9 months after collapse of the lung.

The animals were killed by the intravenous injection of pentobarbital sodium (75 mg per kilogram of body weight). One dog was sacrificed at one month, 4 at two and one-half

months, two at 4½ months and two each at 13, 16, and 17 months after pulmonary collapse was shown to be complete.

Immediately after death the sternum was removed and a ligature placed about the base of the heart to hold the blood which served as an injection medium in the pulmonary vascular system. The lungs were then fixed *in situ* by the intratracheal injection of Zenker-formol solution under low gravity pressure into the unobstructed lung. From 5 to 10 ml of Zenker-formol solution were then injected into the atelectatic lungs through a bronchus peripheral to the point of stenosis. A lobe of a collapsed lung of each animal was expanded with larger amounts of fixative to note the extent of fibrosis. The lungs were then removed *in toto* and placed in Zenker-formol solution for 8 to 12 hours. Thereafter, they were cut, washed, and specimens from both the expanded (right) and collapsed (left) lungs were dehydrated, embedded in paraffin, and sections cut from 10 to 50 μ in thickness. Different sections were then stained with Mallory's azan technique for connective tissue, orcein for elastic fibers, and hematoxylin-eosin-azure II stain for histological detail (Buchsbaum and Loosli, '36).

OBSERVATIONS

Principally as a result of medication and the bronchoscopic procedure the dogs were listless for several hours, but usually returned to normal activity in 48 hours. A cough was provoked and persisted in some animals for several days following application of the silver nitrate. Once bronchial stenosis and atelectasis was complete and the shift of the mediastinum stabilized, coughing ceased. Thereafter, for the duration of the period of observation, the dogs appeared entirely normal. A complete description of the clinical and roentgenographic findings and certain aspects of the gross and microscopic changes in the lungs of dogs following the production of obstructive atelectasis has been made by Adams and Livingstone ('31, '32). In the present study special attention is given to the microscopic findings in the lungs.

Gross appearance of the lungs. In the intact animal the left collapsed lung was seen as a small mass of purplish-red non-crepitant tissue lying posterior to the heart. The right lung, on the other hand, was enlarged and the anterior margins extended beyond the mediastinum into the left chest cavity. The pleural surfaces of the lungs were smooth and showed no adhesions; nor was there fluid in the pleural cavities. There were no blebs on the pleural surfaces indicating emphysema. The expanded (right) and atelectatic (left) lung from a dog killed 4 weeks after the application of silver nitrate are shown in figure 1 for comparison. The relative size of the two lungs was determined by the amount of fluid they displaced when emersed in saline solution. The left lung displaced 55 ml while the saline filled right lung displaced 750 ml. The expanded and collapsed lungs of the animals killed at longer intervals had the same gross appearance as the specimen shown in figure 1.

Microscopic observation of expanded lungs. Although there was a marked compensatory enlargement of the right lung in all the animals, there is no microscopic evidence of emphysema as noted by the destruction, tearing, or thinning of the alveolar walls, even 17 months after collapse of the left lung. The respiratory lobules are clearly demonstrated (fig. 2) and the alveolar walls show no diminution in the number of capillaries (fig. 8). The alveoli are quite uniformly expanded by the fixative. Sections stained with orcein and azan methods show no fragmentation of the elastic or increase in the collagenous fibers. Hypertrophy of the right lung is due to stretching with individual enlargement of the components of the bronchial tree and respiratory lobules (fig. 2).

A conspicuous feature of the alveolar walls is the abundant network of capillaries filled with red blood cells and an occasional leucocyte (fig. 8). The capillary surfaces are smooth and exceedingly thin. The septal cells are clearly seen as isolated cells. They occupy various positions in and on the alveolar walls but are usually seen at the interstices of the septa. Some extend across the alveolar wall so that portions

of cytoplasm protrude into the adjacent alveolar spaces (fig. 8). The attached portion of the cytoplasm of the septal cells rests on and blends intimately with the underlying connective tissue and ground substance of the alveolar walls (fig. 8).

With the hematoxylin-eosin-azure method the nucleus of the septal cell is stained a deep blue. The cytoplasm varies in amount and contains vacuoles of uniform size. It does not blend with or extend over the surfaces of the capillaries to form a continuous covering. The membrane between the capillaries and the alveolar air spaces consists only of the cytoplasm of the endothelial cells and the connective tissue ground substance which also encloses the reticular and elastic fibers.

In thick sections which afford a surface view of the alveolar walls numerous openings or pores are seen in the intercapillary spaces. In thin sections these appear as partial or complete interruptions in the alveolar septa. Occasionally a pigment-filled macrophage is seen in the process of passing through a pore from one alveolus to another.

Microscopic observation of collapsed lungs. Two dogs (no. 9 and 30) killed respectively at 14 and 17 months show evidence of a chronic inflammation. The larger bronchi in the lungs of these animals are filled with mucus and are similar to those shown in figure 3. The mucus contains a moderate number of cells which are principally macrophages. The smaller bronchioles are filled with a cellular exudate in all stages of organization. Around these bronchioles there is a cellular reaction characterized by macrophages which infiltrate the bronchial walls and fill the alveoli. In such areas the septal cells are prominent but in no case do they assume the arrangement of a continuous cellular membrane over the alveolar surfaces.

The lungs of a third dog (no. 17) killed 16 months after application of the silver nitrate show considerable hemorrhage in the collapsed alveolar spaces and fibrosis of the lung tissue about the bronchial stump of the left lower lobe which was removed 9 months after collapse. The red blood cells

and leucocytes in the air spaces appear normal. It was noted in the protocol of this animal that the atelectatic lung was accidentally snipped with scissors and bled "considerably" before it was fixed and removed from the animal. In the scar tissue in the region of the lobectomy scar and in the alveoli about the scar are numerous macrophages filled with pigment, presumably iron, acquired from the ingestion of extravasated red blood cells at the time of left lower lobectomy.

The collapsed lungs of the remaining 9 dogs show no evidence of inflammation or hemorrhage. The microscopic appearance of the atelectatic lungs in these animals is essentially the same as that shown in figures 3 to 7 from the lungs of dog no. 55 killed 17 months after stenosis was produced.

Bronchial tree. The large bronchi are all filled with mucus (fig. 3) and the epithelial cell membrane is intact. The bronchial glands are prominent and extend through the muscle layer into the connective tissue about the cartilage plates. The smooth muscle bands are prominent and the cartilage plates in the walls of many bronchi overlap one another (fig. 4). The bronchial arteries are clearly seen. They are patent and show no evidence of obstruction, sclerosis or thrombosis. The peribronchial lymphatics are also patent. The nerve ganglion and fibers are normal histologically (fig. 4).

The small and terminal bronchioles which do not possess cartilage in their walls do not contain mucus and are collapsed. The cuboidal cell membrane is intact and thrown into folds (fig. 5). Some contain collections of macrophages but usually they are empty.

Pulmonary vessels and lymphatics. The pulmonary arteries and veins are prominent and appear numerous in relation to the collapsed parenchyma. The arteries are distinguished by their deeply staining muscle and elastic fibers in the media (fig. 5). Both arteries and veins are patent, filled with red blood cells, and show no evidence of obstruction, sclerosis, or thrombosis.

The lymphatic channels in the atelectatic lungs are collapsed and less easily distinguished. Small ones occasionally

can be seen in the adventitial coat of the larger arteries and veins and lying adjacent to the collapsed air spaces in the pleura (figs. 4, 5).

Pleura. As a result of collapse the pleura is thickened and occasionally thrown into folds. There is no abnormal cellular infiltration. The mesothelial membrane appears as a single squamous cell layer and the underlying tissue consists of bands of reticular, collagenous and elastic fibers. The relative thickness of the pleura in the expanded and atelectatic lungs may be observed by comparing figures 2 and 5.

Pulmonary parenchyma. The degree of collapse in contrast to the expanded lung tissue may be observed by comparing figures 2 and 5 and figures 7 and 8. The respiratory lobules cannot be distinguished in the collapsed lungs. The alveolar ducts and alveoli appear as slits and crevices in what might be called "solid" tissue (figs. 4-7). The walls of these structures are shortened, thickened, and folded on themselves (figs. 4-7). As seen in the orcein and Mallory-azan preparations the elastic and collagenous fibers are abundant in the alveolar septa. They appear, however, to be no more abundant in the lungs of animals killed at 17 months than in those killed at 10 weeks after collapse.

The capillaries, likewise, are abundant in the alveolar walls and are supported in the meshes of the reticular, collagenous and elastic fibers (figs. 6, 7). As a result of the shortening of the septa the capillaries are tortuous and the intercapillary spaces in some septa are obliterated (fig. 6). As in the expanded lung the wall separating the capillary lumen from the collapsed air space is extremely thin (compare figs. 6 and 7 with 8). The study of sections of the atelectatic lungs stained by the different techniques shows the respiratory membrane to be similar to that seen in the expanded lung. It consists of the endothelial cell cytoplasm, the connective tissue ground substance which encloses the capillaries, and the reticular, collagenous and elastic fibers (figs. 6, 7 and 8).

In areas in which the alveolar surfaces have not been separated by the fixative and are in contact with one another, the atelectatic lung tissue has the appearance of loose connective tissue (fig. 7).

Septal cells. As in the expanded lungs the septal cells (the isolated epithelial cells of older authors) in the atelectatic lobes appear as isolated cells. They vary in number in different parts of the parenchyma but are no more frequently seen in the lungs of the animals killed at 17 months than in the lungs of those killed at 10 weeks. The septal cells assume a variety of shapes and sizes in the atelectatic tissue (figs. 6, 7). In general, the vacuolated cytoplasm of the septal cells is more abundant in the collapsed lung than in the expanded lungs (compare figs. 7 and 8). In no preparation was the cytoplasm seen to extend over the surfaces of the capillaries to form a continuous membrane. In the Mallory-azan sections it can be seen clearly that the septal cells are intimately associated and blend with the ground substance of the alveolar walls. In some preparations the cytoplasm of the septal cells appeared to extend along the central connective tissue stroma of the alveolar walls (fig. 6). Non-nucleated plaques connecting the septal cells into a continuous membrane are not seen.

Re-expansion of collapsed lungs. In spite of the intense and long continued collapse of the left lungs they could be readily re-expanded by the fixative (figs. 9, 10). Lungs re-expanded after 17 months appear no different histologically than do those re-expanded after 10 weeks. There is no fibrosis or obliteration of the normal architecture of the parenchyma. The isolated septal cells could be seen in their characteristic position on the alveolar walls. In no preparation could a membrane composed of these cells be seen covering the alveolar walls (fig. 10). The central connective tissue fibers which support the complex network of capillaries as they bulge into the adjacent alveolar spaces can be observed clearly in the re-inflated lungs (fig. 10).

DISCUSSION

The silver nitrate method of producing permanent obstructive atelectasis of the lungs is a simple and efficient one.

Adams ('30) and Adams and associates ('30, '31, '32, '33, '35) have employed this method extensively in their studies of experimental lung surgery in the dog. Early in their investigations they ('30, '31, '32) found that only stenosis of the main-stem bronchus was followed by atelectasis of a lobe. Because obstruction of secondary bronchi was not followed by collapse of the parenchyma distal to the stenosis, they concluded that there were communications between the smaller air passages such as the alveolar ducts and alveoli, between the walls of adjoining lobules and also between lobes at the hilum. Their observations stimulated Van Allen and associates ('31) to study extensively this "collateral respiration." The communications affording the passage of air through the walls of adjacent lobules were considered to be the pores of Kohn. These structures are present in a wide variety of mammalian lungs including man (Macklin, '36; Loosli, '38). Inter-alveolar communications were numerous in the walls of the hypertrophied uncollapsed right lungs of this series of animals. These openings in the intercapillary spaces provide further evidence for the absence of a continuous cellular membrane lining the pulmonary alveoli (Loosli, '38).

With proper precautions as recommended by Adams ('33) complete collapse of the right or left lung of dogs can be achieved without alteration of the fundamental architecture or histological character of the parenchyma. The pathogenesis of the cellular reaction leading to stenosis of the bronchus at the site of application of the silver nitrate solution has been described by Adams and Livingstone ('31, '32). Van Allen and Adams ('30) studied the mechanism of obstructive pulmonary atelectasis and found that collapse begins first in the region of the hilum and spreads peripherally. They concluded that the pentup bronchial air is lost from the lung through blood stream absorption. Adams and as-

sociates ('35) also made observations on the circulation of blood in the collapsed lung and found it to be greatly diminished. Their studies support the views of Coryllos and Birnbaum ('29) who concluded that "circulation and ventilation of the lung are parallel functions; where ventilation is impaired circulation is decreased and conversely." Henderson ('35) reviews the physiological and clinical manifestations of atelectasis in man.

In spite of the long duration of atelectasis (17 months) in the dogs in this study, sufficient circulation was maintained to preserve without demonstrable fibrosis, the structure of the various components of the lung parenchyma. The inflammatory reaction in the atelectatic lungs of the two dogs in this series may be interpreted as being due to (a) the fact that the silver nitrate swab was too wet allowing the excess solution to run peripherally into the bronchi where it produced a chemical pneumonia; or (b) to a pre-existing low grade infection which was present prior to cauterization of the bronchus and not to collapse *per se*. Although these lungs were not cultured there was no abscess formation or bacteria in the tissues when observed microscopically. The absence of sclerotic changes in the lungs of 9 dogs is indicated by the fact that their atelectatic lungs could be re-expanded readily with the fixing solution. Adams and associates ('35) found that a pressure of 35 to 40 mm of mercury was necessary to reinflate the atelectatic tissue when the air was inserted into the bronchus distal to the region of the stenosis. They also observed that lungs collapsed for several months re-expanded to a lesser degree than did those made atelectatic only for several weeks.

Wolfe and associates ('34) studied compression atelectasis in the dog by enclosing a lobe as far as the hilus in a loosely fitting envelope of thin rubber. This produced an intense pleural inflammatory reaction with resultant fluid formation. The fluid collapsed the lobe and the thickened fibrous pleura produced further shrinking. Atelectasis was so intense that the circulation was impaired. The lung parenchyma then

was converted progressively into a firm fibrous mass, without intervening stages of necrosis and abscess formation. No such changes were noted in this series of animals in which the lung collapsed from its own elastic recoil following absorption of the residual air after stenosing the bronchus.

The blood in the alveolar spaces of the atelectatic lungs of one animal in this series can be explained on the basis of accidental trauma (cutting) of the congested tissue before the blood in the vessels and capillaries had time to clot. Certain vascular changes which were described by Adams and associates ('31, '35) in chronic experimental atelectasis in the dog were not observed in this series of animals. The explanation of this is not readily apparent. The method of producing the atelectasis was the same in the two studies, but the methods of preparation and handling of the tissue were quite different. In the present series of animals great care was taken to fix adequately the pulmonary tissue before it was handled or blocked for sectioning; whereas in the previous study by Adams and associates ('35) the lungs were subjected to considerable handling and surgical trauma. The dogs were sacrificed by electrocution (Hrdina, '29) and the lung tissue for microscopic study was removed immediately after death.

One of the most conspicuous features of the collapsed lungs in this series of animals was the marked vascularity of the parenchyma. With the vascular and capillary bed purposely kept filled with blood, the lungs appeared to be hemangiomatous in character. There was no extravasation of blood, however, into the tissue or air spaces except in the lungs of one dog as described above. Nor was there evidence of intravascular or capillary thrombi. The capillaries in the alveolar walls could be seen clearly in relation to the connective tissue stroma and septal cells. In spite of the marked atelectasis which resulted in a shortening and thickening of the alveolar walls, the respiratory membrane separating the capillary lumen from the air spaces was exceedingly thin. As in the previous study on collapsed lungs of rabbits (Loosli, '35)

a flattened cellular membrane covering the capillaries and continuous with the epithelial cells of the terminal and respiratory bronchioles was not seen in the atelectatic lungs of dogs. Likewise, an alveolar membrane composed of cuboidal cells such as is seen during the glandular phase in fetal lung development was not observed, although Miller ('32, '37) maintained that such a structure makes its appearance following lung collapse.

The septal cells (epithelial cells of older authors) appeared as isolated cells in both the expanded and atelectatic lungs. These observations confirm those made previously by one of us (Loosli, '35, '37, '38) and Hesse and Loosli ('49) employing dogs as well as other animals and methods of study. In the atelectatic lungs the vacuolated cytoplasm of the septal cells appeared to be more abundant than in those along the walls of expanded alveoli, but at no time did it appear to blend with or spread over the surface of the capillaries to form a continuous covering such as can be seen in the lung of the frog which possesses a thin but a histologically demonstrable alveolar epithelial membrane (Loosli, '38; Hesse and Loosli, '49). On the other hand the attached surfaces of the septal cells appeared to blend and be intimately associated with the connective tissue stroma of the septa as has already been described in the lungs of a variety of mammals including man (Loosli, '35, '38, and '48 in Maximow and Bloom's *Textbook of Histology* and Hesse and Loosli, '49).

The origin and nature of the septal cells in the adult mammalian lung have been the subject of much study and discussion, a review of which has previously been made (Loosli, '38). The majority of workers considers them isolated residual epithelial cells (Macklin, '46) or cells of mesenchymal origin (Loosli, '38). On the basis of his study of these cells in the embryo, newborn, and adult mammalian lungs ('38); in the lungs of dogs and monkeys subjected to experimental pneumococcal pneumonia ('42); in the lungs of mice subjected to influenza virus A infections ('49); and in the lungs of dogs subjected to intratracheal injections of dyes and particu-

late matter (unpublished data) Loosli considers them to be the fibroblasts of the alveolar walls which elaborate the connective tissue fibrils and ground substance which supports and enmeshes the capillaries. The present study also supports this view.

These observations do not support the view of Miller ('32, '37) and Bensley and Bensley ('35) that the septal cells are of epithelial origin and form a continuous lining membrane of the pulmonary alveoli. For his evidence Miller resorted to pathological lung tissue and the Bensleys' illustrations are not convincing. Nor are their views supported by other recent investigations on the pre- and post-natal development of the mammalian lungs (see Loosli, '38 and Hesse and Loosli, '49 for references and further discussion). Non-nucleated plaques lining the collapsed alveoli, likewise, were not observed, nor were they seen in the lungs of dogs, rats and mice made acutely edematous by alpha-naphthyl thiourea (ANTU) poisoning (Hesse and Loosli, '49).

At the present time there is no method by which a histologically visible, continuous membrane of endodermal origin covering the alveolar walls in normal mammalian lungs can be demonstrated. Until one is found the presence of such a membrane must be denied.

SUMMARY AND CONCLUSIONS

1. Application of 35% silver nitrate to the main stem bronchus of the lung of the dog is an efficient and simple method for the production of a permanent obstructive atelectasis.

2. The uncollapsed lung enlarges to compensate for the diminished volume of the opposite atelectatic lung. This enlargement is due to simple dilatation of the bronchioles, alveolar ducts and alveoli without emphysematous changes. In the expanded lungs the septal cells are seen as isolated cells and numerous pores are present in the intercapillary spaces of the alveolar walls.

3. Lungs made atelectatic by the resulting stenosis of the bronchus although greatly reduced in size show no fibrosis after 17 months.

4. In the atelectatic lungs the alveolar walls are shortened, thickened, and folded on themselves. The most conspicuous feature is the close network of capillaries supported in a meshwork of reticular, elastic and collagenous fibers and enclosed in a ground membrane of connective tissue origin.

5. The septal cells in the collapsed lung, as in the expanded lung, are conspicuous and appear as isolated cells. They assume a variety of shapes and positions in and on the alveolar walls. In no area is the cytoplasm of these cells seen to extend over the capillary loops to form a continuous membrane.

6. The intimate association of the septal cells with the connective tissue stroma of the alveolar walls strongly points to their mesenchymal origin.

7. Non-nucleated plaques are not demonstrated. The membrane separating the capillary lumen from the alveolar space consists only of the endothelial cells, the supporting reticular fibers and the connective tissue ground substance in which they are embedded.

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PLATE 1

EXPLANATION OF FIGURES

1 Photograph of unfixed lungs of dog (no. 116) 4 weeks after application of 35% silver nitrate to the left main-stem bronchus. The left lung was completely airless, purplish red in color and weighed 55 gm. Stenosis of the bronchus was complete as noted by the failure of the left lung to expand following the intratracheal injection of normal saline. Approximately one-half actual size. (RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; SUBC.L, subcardiac lobe; LLL, left lower lobe; LUL, left upper lobe; TR, trachea.)

2 Low power photomicrograph of a section of expanded right lung of dog (no. 55) whose left lung had been collapsed for 17 months. The lung was fixed intratracheally with Zenker-formol solution and the section shows several respiratory lobules. The alveolar walls are intact but the spaces appear somewhat enlarged. There is no emphysema. (TBR, respiratory bronchiole; ALD, alveolar duct; AL, alveoli; BV, blood vessels; PL, pleura.) Hematoxylin-eosin-azure II stain. $\times 50$.

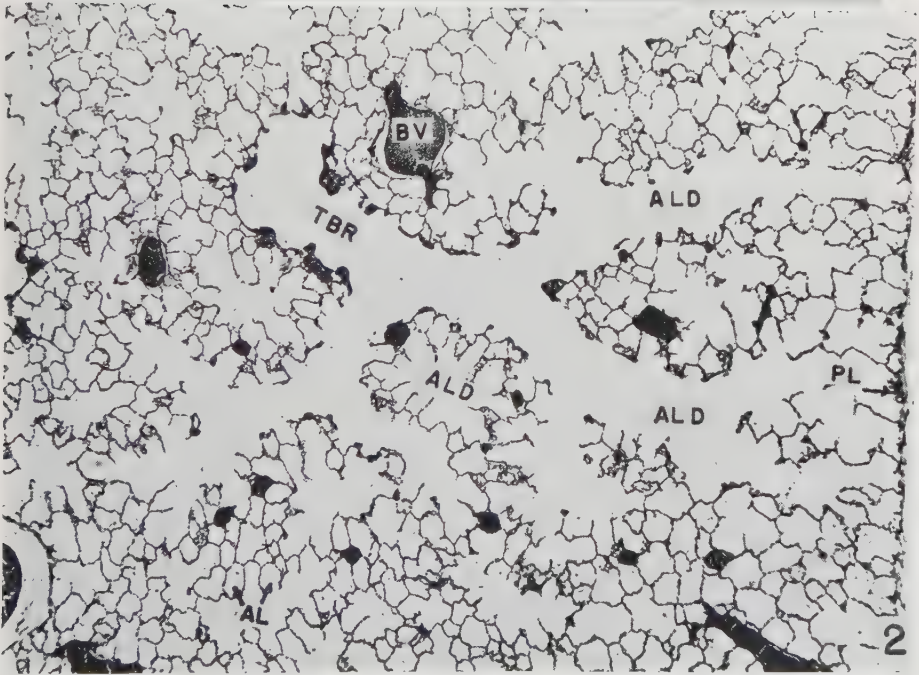
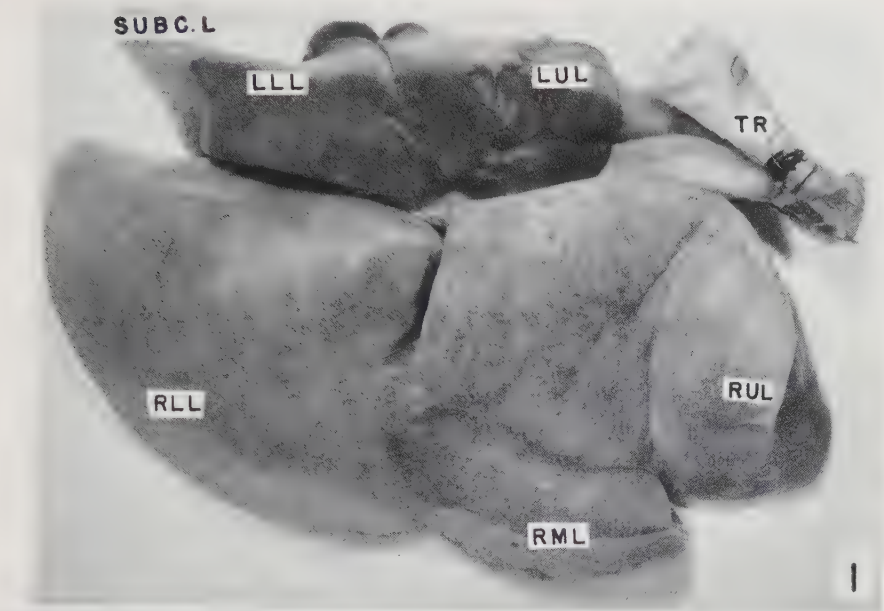


PLATE 2

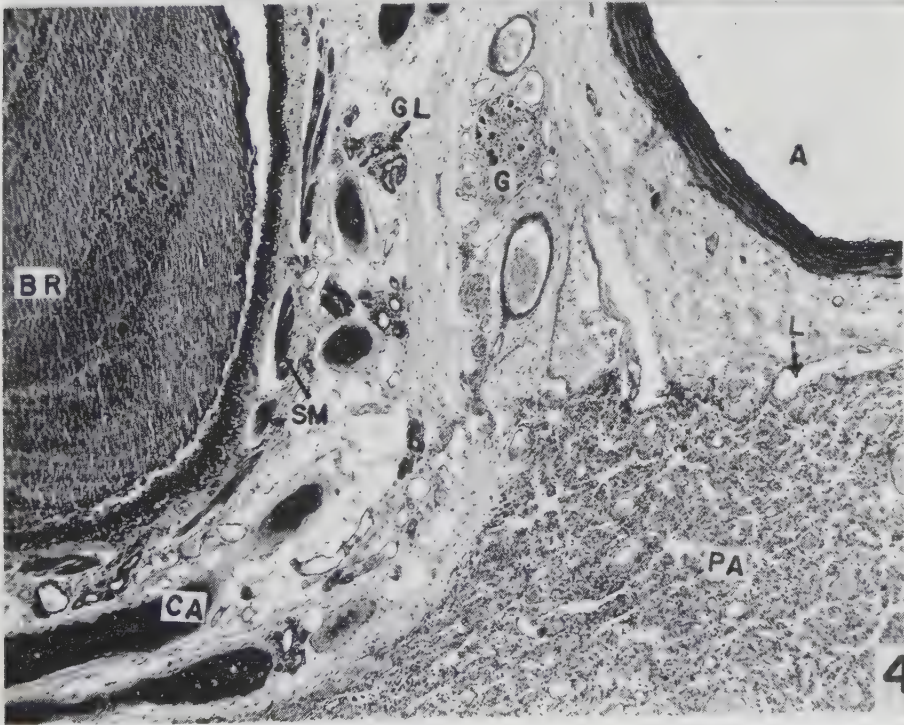
EXPLANATION OF FIGURES

3 Lower power photomicrograph of a section of the left lower lobe of the lung of dog (no. 55). The left lung has been atelectatic for 17 months and appears essentially as a solid organ. The dark staining material in the bronchi is mucus. The blood vessels, filled with blood, are prominent in the collapsed lung parenchyma. (BR, bronchus; A, pulmonary artery; V, pulmonary vein.) Hematoxylin-eosin-azure II. $\times 3\frac{1}{2}$.

4 Medium low power photomicrograph of section near the hilum of left lower lobe of dog (no. 55) killed 17 months after collapse of left lung. There is no evidence of inflammation in the walls of the bronchi blood vessels or lung parenchyma. The cartilage plates of the bronchial wall overlap. A nerve ganglion can be seen in the interstitial tissue. The mucus glands lie between the cartilage plates. (BR, bronchus; A, pulmonary artery; CA, cartilage; G, nerve ganglion; PA, lung parenchyma; GL, mucus gland of bronchus; SM, smooth muscle; L, lymph vessel.) Hematoxylin-eosin-azure II. $\times 60$.



3



4

PLATE 3

EXPLANATION OF FIGURES

5 Medium low power photomicrograph of a section at the periphery of the left lower lobe of dog (no. 55) killed 17 months following collapse of the left lung. The cells in the bronchus are pigment-filled macrophages. There is no evidence of inflammation in the collapsed lung parenchyma or interstitial tissue of the bronchi, blood vessels or pleura. Note the prominence of the blood vessels filled with red blood cells throughout the collapsed lung. The alveolar ducts and alveoli appear as narrow slits. (BR, bronchiole; A, artery; V, vein; PL, pleura.) Hematoxylin-eosin-azure II. $\times 60$.

6 Oil emersion photomicrograph of a section of left lower lobe of dog (no. 55) killed 17 months following collapse of the left lung. The alveolar spaces have been separated slightly by the fixing fluid. The alveolar walls are shortened, thickened, and folded on themselves. The capillaries are prominent and filled with red blood cells. The intercapillary spaces are narrow or absent. The septal cells are not conspicuous and blend with the connective tissue stroma. They are not seen to cover the capillaries as a continuous membrane. (AL, alveolus; C, capillary; S, septal cell.) Hematoxylin-eosin-azure II. $\times 1000$.

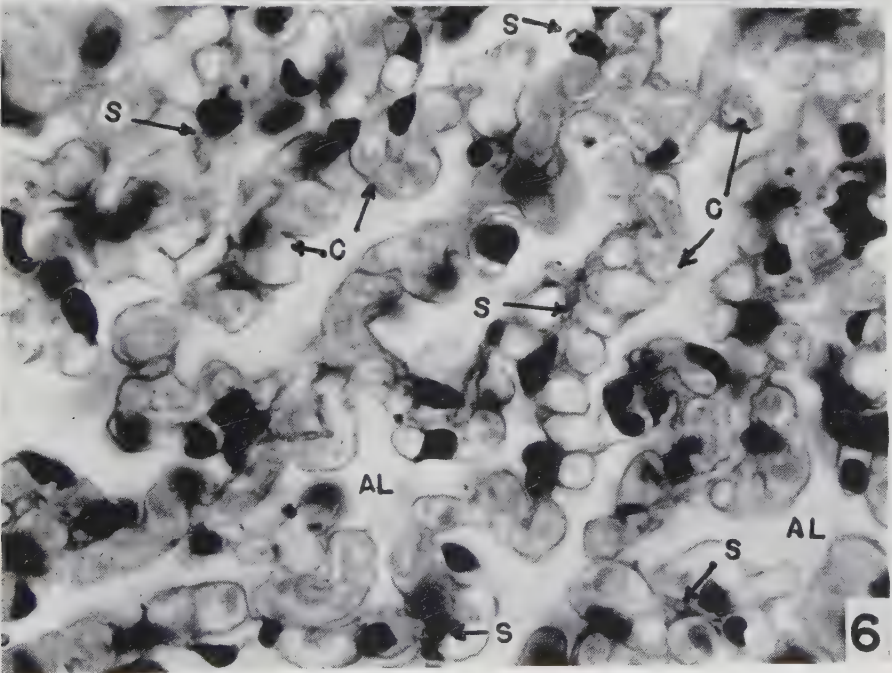
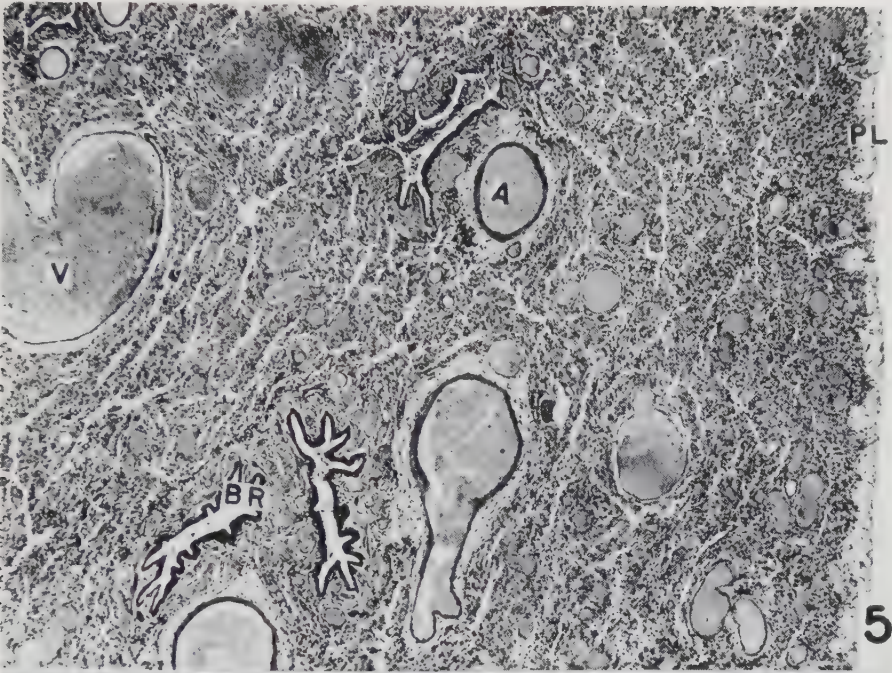


PLATE 4

EXPLANATION OF FIGURES

7 Oil emersion photomicrograph of a section of the left lower lobe of dog (no. 22) sacrificed 14 months after collapse of the left lung. The majority of alveoli are completely collapsed or appear as slit-like spaces. The lung tissue resembles loose connective tissue. The capillaries are abundant, patent, and their surfaces are smooth and thin. The septal cells appear as isolated cells with abundant foamy cytoplasm. The cytoplasm of the septal cells does not cover the surface of the capillaries. The membrane separating the capillary lumen from the air space consists only of the endothelial cell cytoplasm and the ground connective tissue substance which encloses the capillaries as well as the connective tissue fibers. (BR, respiratory bronchiole; AL, alveolus; C, capillary; END, endothelial cell; S, septal cells.) Hematoxylin-eosin-azure II. $\times 1060$.

8 Oil emersion photomicrograph of right lower lobe of dog (no. 22) sacrificed 14 months after collapse. The capillaries are filled with red blood cells. The septal cells are located in their characteristic positions in and on the alveolar walls and are intimately associated with the connective tissue stroma of the septa. Some septal cells lie across the septum and protrude into the adjacent alveolar spaces. The cytoplasm does not spread over the surface of the capillaries to form a continuous membrane. (AL, alveolus; C, capillary; END, endothelial cell; MAC, macrophage; S, septal cell.) Hematoxylin-eosin-azure II. $\times 1060$.

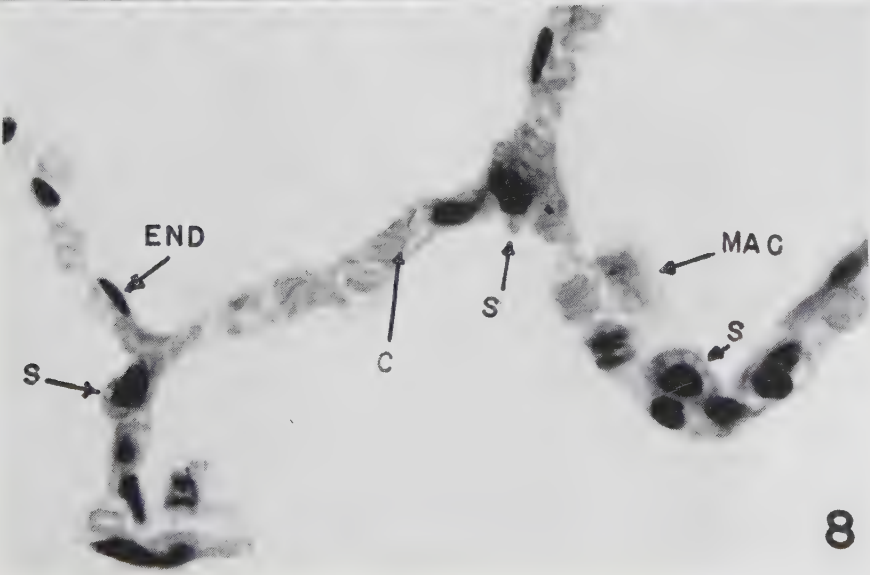
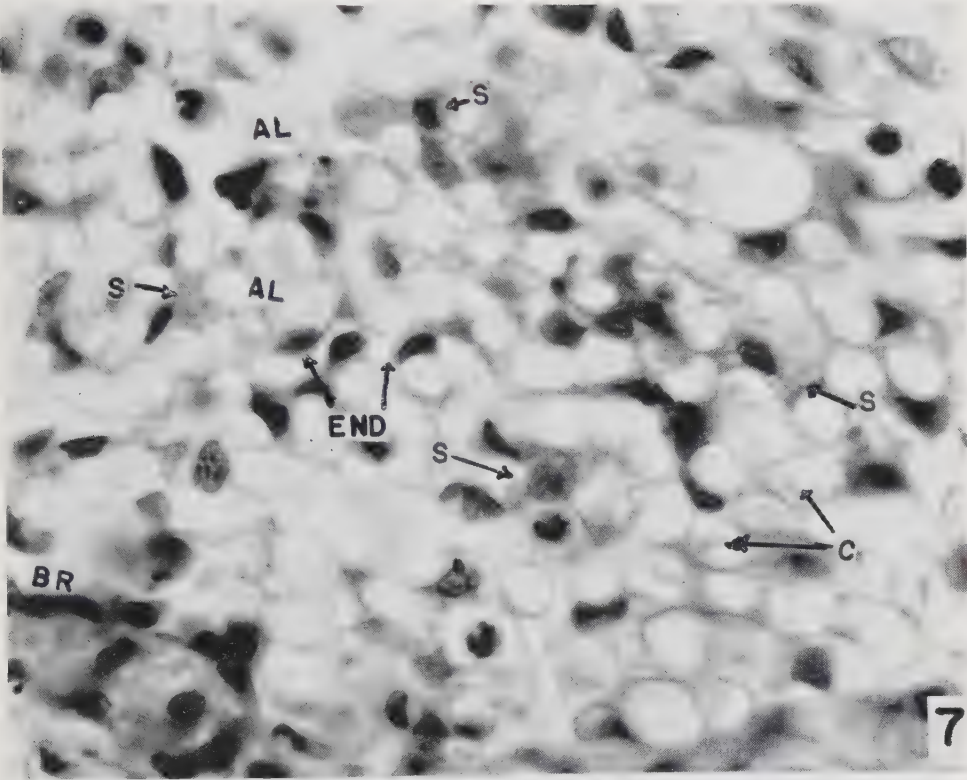


PLATE 5

EXPLANATION OF FIGURES

9 Medium low power photomicrograph of a section of the left lower lobe of the lung of dog (no. 12) sacrificed 16 months after collapse of left lung. The lung was re-expanded with fixing solution. There is no apparent fibrosis or alteration of the architecture of the lung parenchyma as a result of collapse. There is no evidence of inflammation. (A, artery; AL, alveolus; ALD, alveolar duct; BR, bronchiole; PL, pleura.) Hematoxylin-eosin-azure II. $\times 60$.

10 Oil emersion photomicrograph of re-expanded lung shown in figure 9. The capillaries are patent, tortuous and filled with red blood cells and leucocytes. The septal cells blend with the connective tissue stroma of the alveolar walls. The capillaries surfaces are thin, smooth and free of an epithelial covering. (AL, alveolus; C, capillary; END, endothelial cell; MAC, macrophage; S, septal cell.) Hematoxylin-eosin-azure II. $\times 950$.



CALCIFICATION IN THE FETUSES OF NORMAL AND ANCON SHEEP

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TEN FIGURES

Our previous studies on Ancon sheep have shown that this mutation is inherited as a simple recessive and that the dwarfing is a type of chondrodystrophy (Landauer and Chang, '49; Chang, '49a, '49b, '49c). It has been shown that the epiphyseal plates of the newborn lambs are perforated and disintegrated to various degrees and this is closely related to the extent of shortening of the bones. In hematoxylin-eosin preparations the matrix of the cartilaginous patches lodged in the network of blood vessels gave a much deeper blue stain than it does normally. The hematoxylin stain in cartilage often is considered as due to the calcification of the cartilage matrix. This phenomenon suggested that a study of the calcifications of the cartilage in normal and Ancon embryos would be of interest. Calcification of cartilage in the normal rat has been studied and the reaction of calcification to hematoxylin stain fully discussed by Bloom, McLean, et al. ('40).

Since Robinson's biochemical study of ossification ('24), it has been generally accepted that mineralization in cartilage and bone is related to alkaline phosphatase. He further demonstrated a higher content of phosphatase in rachitic bones. The phosphatase content of the bones of Creeper fowl (Lan-

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dauer et al., '35) and of the blood serum of chondrodystrophic man (Bodansky and Jaffe, '34) were known to be normal. The localization of the glycerophosphatase in demineralized bones of various kinds of animals at different stages has been investigated (Moog, '46; Lorch, '47; Greep and Morse, '48, and Zorzoli, '48). As the cartilage in the Ancon sheep is hypoplastic, it was also interesting to see whether or not there is a difference in alkaline phosphatase.

The onset of ossification centers in the sheep fetus was studied first by Harris ('37) and later by Andreeva ('40). The radius was found to begin to ossify on the 40th day and the third and 4th metacarpi on the 48th day. The 46th day of development of sheep fetuses was chosen for our study.

MATERIAL AND METHODS

One heterozygous (normal) and three homozygous (Ancon) fetuses of 46 days were removed from the uteri, photographed and then immediately subjected to the following procedures:

1. The right fore- and hindlegs were fixed in Heidenhain's Susa for hematoxylin-eosin stain.
2. The left forelegs were prepared according to Bloom's modification of Kossa's method ('40) for calcification study and counter-stained with hematoxylin-eosin-azure.
3. The left hindlegs were fixed in chilled acetone according to Danielli's ('46) recommendation of Takamatzu-Gomori's alkaline phosphatase technique, but sodium barbital was used instead of sodium veronal. Sections were incubated 9 hours in sodium glycerophosphate (Eastman Kodak no. 644) as substrate. Control sections were incubated without the substrate.

OBSERVATIONS AND DISCUSSION

The total length of the 46-day normal and Ancon fetuses is 62.5 mm and 61 mm respectively. The shortening of the legs in Ancon fetuses can be shown by the graphic reconstruction of the radius and metacarpus (fig. 1).



Fig. 1 Graphic reconstruction of radii and third metacarpi of 46-day fetuses. $\times 10$. FC—flattened cell columns, HC—hypertrophied cell columns, PC—primary ossification center, MC—marrow cavity.

- A. Radius of the normal fetus.
- A'. Radius of the Ancon fetus.
- B. Third metacarpus of the normal fetus.
- B'. Third metacarpus of the Ancon fetus.

Microscopically, the chondrocytes in the Ancon bones are normal in arrangement and structure, but the matrix in the hypertrophied zone is more extensive (fig. 2, A-B). Striking differences can be revealed by measurements.

Table 1 shows that both in normal and Ancon fetuses the cell columns of the cartilage, the periosteal bone outside

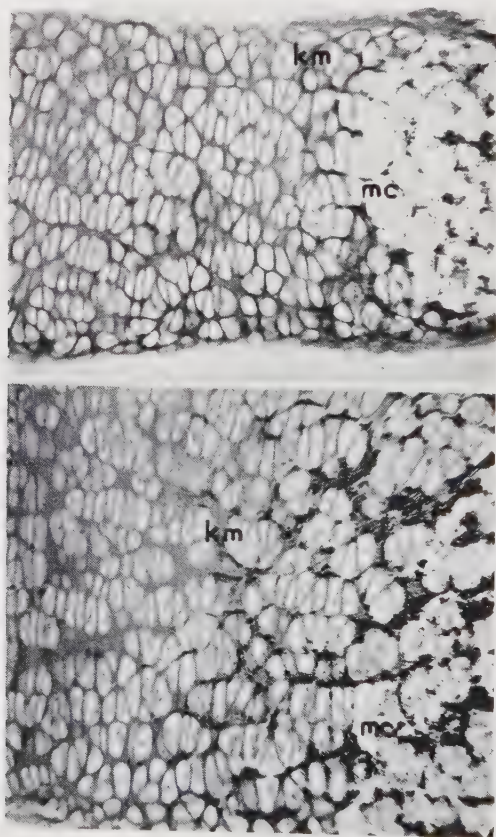


Fig. 2 Longitudinal section of the distal end of radius. Silver nitrate and hematoxylin-eosin-azure stain. $\times 100$.

km — calcified matrix mc — marrow cavity

Above: Magnification of the distal end of normal radius.

Below: Magnification of the distal end of Ancon radius.

TABLE 1
The following data are the averages in micra of each 5th section of the serial sections of the distal and proximal ends of the radius

	FLATTENED CELL COLUMNS	HYPERTROPHIED CELL COLUMNS	CALCIFIED MATRIX	OSTIFIED LENGTH	PERIOSTEAL BONE THICKNESS	PERIOSTEUM	
						Length	Thickness
<i>Proximal end</i>							
Normal radius	691	317	84 (4.5 cells)	154	11	16	43
Ancon radius	531	322	146 (10 cells)	266	14	19	37
<i>Distal end</i>							
Normal radius	938	502	67 (4 cells)	163	15	17	51
Ancon radius	669	410	231 (15 cells)	375	19	21	46

of the cartilage and the osteogenic tissue of the distal end are longer and thicker than at the proximal end. This is in agreement with the events of postnatal growth of this bone as observed in pig (Payton, '32) and man (Bergmann, '31); that is, the distal epiphyseal plate of the radius contributes more to the growth in length than does the proximal one. In comparison with the normal bone, the cell columns at both ends of the Ancon radius are reduced in length, particularly the flattened cell columns. On the other hand, the length and thickness of the periosteal bone and, to a lesser extent, the thickness of the osteogenic tissue, is more extensive. This is in agreement with our previous observations on postnatal growth: the Ancon sheep has more extensive periosteal ossification.

Calcification. The length of the area covered by the calcified matrix in the normal is, on the average, $84\ \mu$ (4.5 cells) in the proximal end and $67\ \mu$ (4 cells) in the distal. That of the Ancon radius is $146\ \mu$ (10 cells) and $231\ \mu$ (15 cells), respectively. The situation in the normal bone is in accord with the observations of Bloom et al. ('40) in the rat; that is, it generally covers two to three cells and rarely 7-8 cells. In considering the difference in the width of the hypertrophied zone in the two kinds of bone it was found that the calcified area in the Ancon radius covers nearly half of the zone while that of the normal one covers only about one-fifth. The silver nitrate technique obviously demonstrated that the calcification of the Ancon cartilage is more extensive. However, the coarse granules of cobalt sulfide which are precipitated by the calcium salts in the bone and cartilage (Zorzoli, '48) in our control sections which were stained for alkaline phosphatase cover most of the hypertrophied zone of both the normal (figs. 4, 6, 8, 10) and Ancon radii (figs. 3, 5, 7, 9). (See table 2.)

The calcified area is more extensive in the normal than in the Ancon because the former has a wider hypertrophied zone. It seems that the metallic silver and cobalt sulfide in the two kinds of preparations are precipitated by different salts.

Alkaline phosphatase. By using the intensity of blackening as criterion, the localization of alkaline phosphatase in the hindleg of both normal and Ancon fetuses is as follows:

1. The enzyme is most concentrated in the endothelial cells of the blood vessels; the osteoblasts and other embryonic cells in the bone marrow; the outer layer of bony trabeculae and their embedded osteocytes; the cells of the periosteum and of the diaphyseal side of the perichondrium; nuclei of the chondrocytes in the articular surface and in those adjacent to the positively stained perichondrium; and the cartilaginous matrix and chondrocytes in a transitional region between the flattened and hypertrophied cell columns.

TABLE 2

The measurements are as follows:

	CALCIFIED MATRIX	LENGTH OF PERIOSTEAL BONE OUTSIDE OF CARTILAGE
	μ	μ
<i>Proximal end</i>		
Normal tibia	497	560
Ancon tibia	314	394
<i>Distal end</i>		
Normal tibia	446	566
Ancon tibia	126	278

2. The next in order of blackening are the nuclei of the skeletal muscles, the fibrous tissue of the periosteum, perichondrium and blood vessels and the chondrocytes in the hypertrophied zone.

3. The stain is least concentrated in the fibrils of the skeletal muscles, the osteocytes and matrix of the older bone spicules and the cartilage matrix of the hypertrophied zone.

Within the cells the enzyme is most concentrated in the nucleus and the peripheral cytoplasm. The perichondrium and cartilage matrix in the distal end of the tibia is more black than at the proximal end, which further proves its more extensive growth. This is in agreement with Roch and Leandri's ('35) quantitative analysis of phosphatase in the long

bones of growing rats. They found the content of phosphatase in the distal epiphysis to be higher than in the proximal one. The metatarsus at this stage of development has the periosteal bone around the shaft and a well developed primary ossification center in the middle, but no erosion has yet appeared in the subperiosteal region. The localization of calcified matrix and alkaline phosphatase follows the same pattern as in the tibia. The primary ossification center is calcified but, as in the hypertrophied zone of other bones, it is only slightly blackened by the phosphatase reaction. At the periphery of this center one finds the deeply stained phosphatase, which is equivalent to that of the transitional region of the tibia.

The above observations on undecalcified bone of sheep fetuses are in close agreement with previous investigations on demineralized bones of various mammals (Lorch, '47; Greep and Morse, '48, and Zorzoli, '48). However, our results have a striking difference from the previous reports in that the most intense localization of the glycerophosphatase is in the chondrocytes and matrix of the distal ends of the flattened cell columns and the proximal ends of the hypertrophied cell columns where the matrix has not yet calcified. It is much less intense in the calcified or hypertrophied zone where previous authors found their greatest concentration. Lorch noticed that the concentration of phosphatase in the hypertrophied zone of the proximal end of the kitten femur is low in comparison with that in the bone marrow of the same bone and the hypertrophied zone in rats and mice. Her kitten material, on the other hand, failed to show the concentration of enzyme in the transitional zone. Whether these differences are due to heterogeneity in material or in preparation needs further investigation. Judging by the sections stained in hematoxylin-eosin-azure, the transitional region belongs mainly, if not entirely, to the flattened zone because the matrix here takes the same bluish tint as the flattened cell columns, rather than the purplish color of the hypertrophied columns. Since the cells in these two zones are continuous and have no sharp

boundary, the staining of the matrix seems to be the only criterion for the arbitrary division. Zorzoli stated, in regard to the chronological appearance of the phosphatase, that "the appearance of enzyme always preceded the appearance of bone salts." This can be changed, when related to our regional observations, to read, "the appearance of the enzyme always preceded the appearance of calcium salts in the cartilaginous matrix."

From figures 3-10 one can see that the transitional region in the Ancon bone is more intensely blackened than the same region of the normal one. It is, however, narrower because of the shorter cell columns. Landauer, Upham and Rubin ('35) have shown that the chondrodystrophic bones of Creeper chickens do not differ from normal ones with regard to their relative phosphatase content. Bodansky and Jaffe ('34) found the serum phosphatase in the blood of human chondrodystrophics to be normal. Our evidence indicated that alkaline phosphatase has no part in the causation of chondrodystrophy of Ancon sheep. It was the hypoplasia of the proliferating cartilage that shortened the cell columns and, concomitantly, retarded the rate of ossification.

SUMMARY

One heterozygous (normal) and three homozygous (Ancon) sheep fetuses, 46 days old, were used for a study of general differentiation, calcification of the cartilage and localization of alkaline phosphatase in the leg bones.

The cartilage in the Ancon fetus is histologically normal but the length of the bone and of the cell columns is shortened, particularly the flattened cell columns. On the other hand, there is indication that the periosteal ossification in the Ancon fetus is intensified.

The silver nitrate test for calcification of cartilage shows that the hypertrophied zone is more extensively calcified in the Ancon fetus. In the control sections after alkaline phosphatase technique, the precipitation in the hypertrophied zone is more extensive in the normal than in the Ancon fe-

tuses. The distinction is attributed to a difference in the kinds of salts in the cartilage.

In alkaline phosphatase preparations the transitional region between the flattened and hypertrophied cell columns is more extensively blackened than the hypertrophied zone. The aforesaid region in the Ancon bone is darker but narrower than that of the normal bone. Alkaline phosphatase does not seem to be causally related to chondrodystrophy in these sheep.

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PLATE 1

EXPLANATION OF FIGURES

3-10 Showing the localization of alkaline phosphatase in the distal end of normal and Ancon tibiae.

fz — flattened zone; hz — hypertrophied zone; mc — marrow cavity

3 Control section of the normal tibia showing calcification of the cartilaginous and osseous tissue. $\times 49$.

4 Adjacent section of figure 3, incubated in a substrate of sodium glycerophosphate for 9 hours, showing the localization of alkaline phosphatase. $\times 49$.

5 Control section of the Ancon tibia showing calcification of the cartilaginous and osseous tissue. $\times 49$.

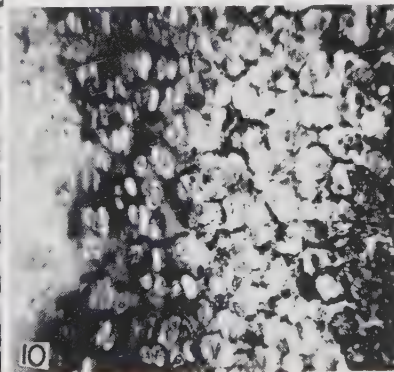
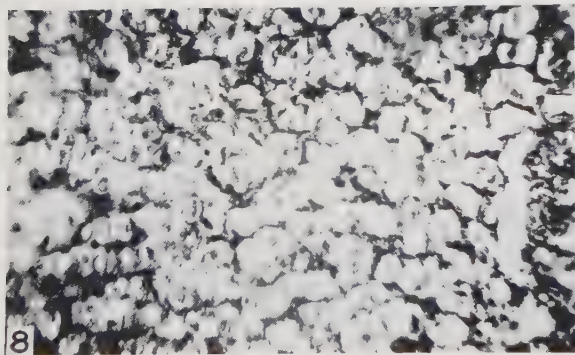
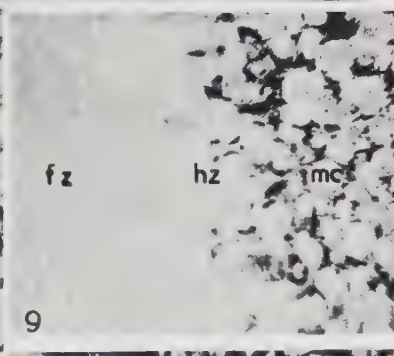
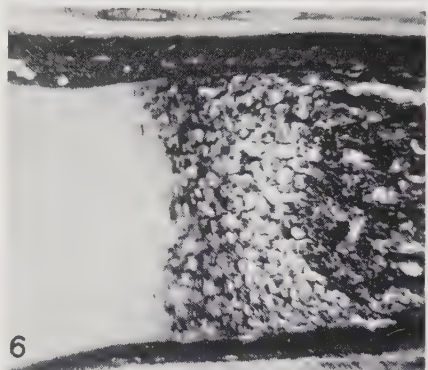
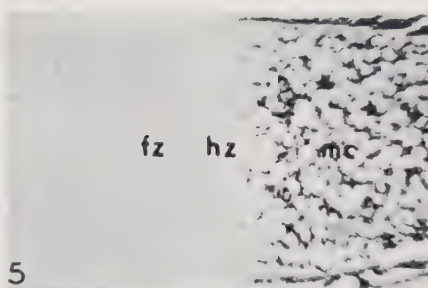
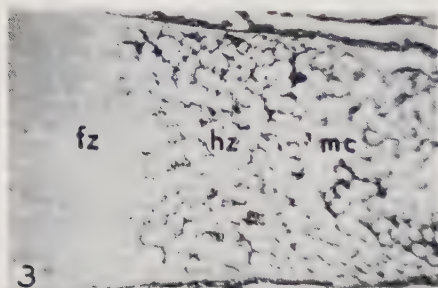
6 Adjacent section of figure 5, incubated in a substrate of sodium glycerophosphate for 9 hours, showing the localization of alkaline phosphatase. $\times 49$.

7 High magnification of figure 3. $\times 100$.

8 High magnification of figure 4. $\times 100$.

9 High magnification of figure 5. $\times 100$.

10 High magnification of figure 6. $\times 100$.



OSSIFICATION AND GROWTH OF THE HUMAN MAXILLA, PREMAXILLA AND PALATE BONE

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SIXTEEN FIGURES

INTRODUCTION

The term maxilla, as generally used in human anatomy, corresponds to the premaxilla and the maxilla in lower animals. All mammals, with the exception of the bats and certain edentates, possess premaxillary bones which generally remain distinctly separated from the maxilla on the facial aspect (Walter, '39). Ever since the publication of Vesalius' *De humani corporis fabrica* in '1543, there has been controversy over the premaxilla in man. It is generally understood that the classical teaching of the anatomy of Galen before Vesalius was based on the anatomy of monkeys. Vesalius denied the existence of the premaxillary suture on the facial aspect of man as was described by Galen, but he noticed the presence of the palatine portion of the premaxillary suture on the palate. Columbus (1559), Fallopius (1561), Kölliker (1882), Fawcett ('11) and others also observed the palatine portion of the suture. Albinus (1746), Goethe (1786) and Rosenmueller (1804) described the endofacial portion of the premaxillary suture. Camper (1778) emphasized the absence of the suture on the facial aspect. Blumenbach (1776), who named the premaxilla as the intermaxillary bone, recognized its human distinction. Wood Jones ('29, '47) regarded the early facial absence of the premaxilla as a "specific human

character." Lawrence (1838) and Rousseau (1858-59) even went so far as to consider it to be completely absent in man. However, Vicq d'Azyr (1780), Meckel (1832), Leuckart (1840), Leidy (1849), Callender (1869), Ranke ('01), Felber ('19), Jarner ('22), Augier ('31) and many others observed the premaxilla in human fetuses. Vicq d'Azyr and Goethe pointed out further its structural equivalence to the same bone in other mammals.

Callender (1869) was the first who gave a detailed developmental account of the premaxilla in man. He examined 20 embryos and fetuses ranging in age from two to 7 months of age and described the premaxilla as being "shut off from the face by the nasal and incisor processes of the superior maxilla" (p. 168). In other words, the premaxilla is enclosed anteriorly by the nasal and incisor processes of the maxilla and for this reason no trace of the bone can be seen on the face even though its presence is assured by a fissure in the palate. This view was denied by Fawcett ('11), but supported by Vallois and Cadenat ('24) and Ashley-Montagu ('35). In addition, Nesbitt (1736), Hamy (1868), Ranke ('01), Vallois and Cadenat ('24), Ashley-Montagu ('35) and Friant ('46) noticed a portion of the premaxillary suture on the facial aspect of human fetuses. Ashley-Montagu ('35) compiled a thorough review of the literature on the subject and made a comparative study of the bone in question in the primates.

There is now agreement that the premaxilla is an element in the human skull and that it remains separate from the maxilla in the palatine portion. However, the details of its structure and the process of its early obliteration on the facial aspect are not yet well understood.

From how many centers of ossification the human maxilla originates has been disputed for more than a century. Between 1840 and 1944 there have been reported by different workers from two to 9 centers.

Leuckhart in 1840 first reported that each premaxilla arises from two ossification centers. Albrecht (1882), Biondi (1888) and Ranke ('01) asserted the same view. Rambaud and Re-

nault (1864) described 6 centers of ossification for the maxilla (two for the premaxilla and 4 for the maxilla proper).¹ Fawcett ('11) stated that the maxilla proper is developed from one center and the premaxilla from two centers. Frassetto ('14) ascribed as many as 5 centers to the premaxilla and 4 centers to the maxilla proper. On the other hand, Kölliker (1888) observed only one center for each premaxilla. Mall ('06), Vallois and Cadenat ('24) and Augier ('31) observed only two centers for the maxilla (one for the premaxilla and one for the maxilla proper). Recently, Noback ('43) stated that the maxilla arises from two centers and one line. He did not explain whether the line was developed from one or more centers of ossification.

From this it may be seen what confusion exists. This confusion has been reflected in textbooks of human anatomy. Piersol ('36) describes 4 centers for the maxilla (one for premaxilla); Cunningham ('43), three centers (two for premaxilla); Morris ('42), 5 centers (two for premaxilla) and Gray ('48), two centers (one for premaxilla).

Rambaud and Renault in 1864 described 4 ossification centers for the palate bone. According to Frassetto ('14) it is ossified from 5 centers. However, Mall ('06), Fawcett ('06) and Augier ('31) stated that the palate bone is developed from a single center.

The morphological changes of the palate bone and its relation to the maxilla are not yet clearly known. Woo ('48a) recently reported the overlapping condition of the palatine processes of the maxillae and of the horizontal parts of the palate bones.

Studies of the developmental anatomy of the human skeleton in the last century were based primarily on observations of grossly dissected embryos and fetuses and on sectioning

¹Augier ('31) and others cited Rambaud and Renault as giving 5 centers to the maxilla. However, Rambaud and Renault described 6 centers in their book: "Le maxillaire supérieur est formé de 6 pièces principales. 1° Une incisive, 2° Une palatine, 3° Une faciale, 4° Une lacrymale, 5° Une malaire, 6° Une sous-vomerienne" (p. 157).

and reconstruction. Schultze (1897) first used KOH in clearing embryos. Lundvall ('05) and Spalteholz ('14) devised the method of staining the cleared specimens with alizarin red S. Dawson ('26), Hollister ('34), Noback ('44) and others modified and improved the technic in using KOH for clearing and alizarin red S for staining the bone. In the last two decades the clearing-staining method has been used to a certain extent. However, no extensive work has been done on this problem. In addition to the technique, the acquisition of a series of human embryos is difficult.

This work is a study of the process of ossification, the formation and eventual obliteration of the facial premaxillary bones, the growth of the premaxilla, maxilla and palate bone and their relationship.

MATERIAL AND METHOD

The material of this study includes 84 human embryos and fetuses ranging in age from 5 weeks to full term obtained through the courtesy of Dr. W. M. Allen of the St. Louis Maternity Hospital and Dr. J. A. Saxton, Jr. of the St. Louis City Hospital. In addition, 34 skulls ranging in age from birth to maturity and 319 sagittally sectioned adult skulls were observed. The age of embryos and fetuses was determined by the C-R or crown-rump length according to Patten ('46). In measuring the older fetuses, the C-H or crown-heel dimension was also determined for the estimation of age.

The method used for clearing and staining embryos and fetuses generally followed the procedures suggested by Noback ('44) with minor modifications. Embryos and fetuses under 4 months of age were eviscerated and decerebrated by dissection. In fetuses over 4 months, the portion with the bones in question was cut off. The specimen was fixed in 75% alcohol for one week to two months according to its size, bleached in H_2O_2 , cleared and stained simultaneously in an aqueous solution of KOH (from 2% to 10% depending upon the size of the specimen) and .01% to .05% alizarin red S until the soft tissues were completely cleared and the bones

properly stained a deep red color. The specimen was then passed through increasing concentrations of glycerin and finally stored in pure glycerin.

OSSIFICATION CENTERS OF THE MAXILLA AND THE PALATE BONE

The maxilla as a whole is found to be developed from three ossification centers on each side; one gives rise to the maxilla proper and the other two give rise to the premaxilla. In no

TABLE 1
Times of appearance of the cephalic ossification centers

NO.	C-R LENGTH	AGE	OSSIFICATION CENTERS PRESENT
	<i>mm</i>	<i>weeks</i>	
80	10	5	none
26	12	6	none
52	12	6	none
44	15	6	none
51	15.5	6	man. ¹
55	16	6	man.
31	18	6	man., max.
49	21	7	man., max., prem.
53	27	7	man., max., prem., pal.
Series ²	40	8.5	man., max., prem., pal.
47	62	10	man., max., prem., pal., inf.

¹ This and the succeeding abbreviations indicate as follows: man., mandible; max., maxilla; prem., main premaxillary center; pal., palate bone; inf., infra-vomerine center of premaxilla.

² This constitutes an average derived from 8 embryos each 8 or 9 weeks of age.

case were more than three centers on a side observed for the entire maxillary area. The times of appearance of the ossification centers of the maxilla proper, the premaxilla, and the palate bone are listed in table 1. It may be seen that the maxillary center first appears in an embryo of 18 mm C-R length (fig. 2) and the main premaxillary center, in an embryo of 21 mm C-R length (fig. 3). Vallois and Cadenat ('24) mentioned that the premaxillary center appears in an embryo of 20 mm C-R length and Augier ('32) stated that it appears

in an embryo of 26 mm C-R length. Fawcett ('11) found that the maxilla proper commences to ossify at about the 18 mm stage. Mall ('06) reported that the ossification center of the maxilla proper appears in an embryo of 15 mm C-R length, estimated to be 39 days old and the premaxillary center, in an embryo of 18 mm C-R length, estimated to be 42 days old. According to Patten's table of fertilization age ('46, p. 184), the embryo of 18 mm C-R length in the present study is about 48 days old and that of 21 mm C-R length is about 51 days old. The results given here show a later date of appearance of the centers of the two bones than those given by Mall. However, these differences are exaggerated by the different methods used in estimation of the age of embryos. The age of Mall's specimen was estimated in days by multiplying the square root of the C-R length in millimeters by 10. In the present study, the age was determined according to Patten's table. Mall's specimen of 15 mm C-R length in Patten's table gives an age of about 45 days, and that of 18 mm C-R length, 48 days. Thus the actual difference in the dates of appearance of the ossification centers of the premaxilla and the maxilla proper in both studies is only three days. It is interesting to note that in both studies the premaxillary center appears three days later than the maxillary center.

Although Mall ('06), Fawcett ('11), Vallois and Cadenat ('24) and Augier ('31) all asserted that the premaxilla and the maxilla proper arise from one ossification center respectively, none were able to demonstrate their independence in an embryo. After failure in many cases Augier in 1932 succeeded in showing the separation of the two centers in one specimen, but he questioned the normality of the fact. In the present study, the two centers in specimen no. 31 have already fused but specimen no. 49 shows clearly the complete independence of the main premaxillary and the maxillary centers (fig. 3). The difficulty in showing the complete separation of the two centers lies in the fact that the premaxillary center unites almost immediately after its appearance with the maxillary center.

The accessory premaxillary center arises in an embryo of 62 mm C-R length (fig. 5). It is estimated to be 75 days. This center forms the infravomerine process of Rambaud and Renault. In the other three embryos of this stage, this process was found to be united with the medial portion of the palatine process of the premaxilla. The fact that the ossification center arises in the immediate neighborhood of the medial end of the horizontal lamella and unites with it very shortly after its appearance may explain why so many authors failed to see it as a separate center of the premaxilla.

The palate bone is developed from a single center of ossification. As shown in table 1, the center of the bone first appears in an embryo of 27 mm C-R length (fig. 4), estimated to be about 55 days. Mall ('06) described the center of the palate bone appearing in an embryo of 33 mm C-R length, estimated to be 57 days. According to Patten's table, the fertilization age of this specimen is about 66 days. Thus, the result of the present study concerning the time of appearance of the ossification center of the palate bone is 11 days earlier than that given by Mall.

GROWTH OF THE MAXILLA

The premaxilla originates "in membrane" on the external surface of the nasal capsule. It arises in a position above the primordium of the lateral incisor tooth at the beginning of the 7th week. A little later it extends upward, sharing with the maxilla proper in the formation of the frontal process. It extends, also, backward, joining the maxilla proper. At the 8th week the premaxillary bone becomes denser in structure and begins to unite with the maxilla proper along the alveolar border. However, the frontal processes of the two bones remain separated for a long time (figs. 6 and 7). At a slightly later stage the maxilla proper extends forward and embraces the premaxillary bone. This extension gradually occurs from the alveolar border upward to the apex of the frontal process and at about the end of the third month, the whole premaxilla is covered by the maxilla proper on the fa-

cial aspect (fig. 8). However, the separation of the two bones may be seen partially on the facial aspect even after birth. Ashley-Montagu ('35) reported a case of a male Negro fetus aged 9.27 months in which the apical portion of the premaxilla was displayed upon the facial aspect. On the palatine and nasal surfaces the premaxilla remains separated from the maxilla proper for a long time. According to Ashley-Montagu ('35) the premaxillary suture may be seen endofacially, to a greater or lesser extent, in some 26% of cases in skulls exceeding 6 years of age. Generally, the premaxilla carries the two incisor teeth except on the facial aspect where it is shared in a small part by the maxilla proper. However, it was observed in several cases of this series that the premaxilla carried the canine tooth as well as the two incisors.

The palatine portion of the premaxilla is more complicated and more easily confused. The bony lamellae are generally arranged in a transverse direction, or more exactly, in an antero-medial and postero-lateral direction (fig. 9). The center of the infravomerine process of Rambaud and Renault appears at the end of the 10th week in an antero-posterior direction and then unites immediately with the medial end of the lamella. The line between the lamella and the longitudinal infravomerine process is the so-called interincisive suture. It may occasionally be observed even in adult skulls (fig. 12). The portion anterior to the suture is called by Albrecht (1882) the endognathion and that posterior to the suture, the mesognathion. It is interesting to note that in alveolar harelip the cleft usually passes between the two portions. The infravomerine process of the premaxilla is characterized by its sagittal orientation whereas the rest of the palatine process is generally horizontal (figs. 10 and 11). The palatine process (including the infravomerine process) extends backward almost to the vomer in forming the anterior portion of the boundaries of the incisive foramina and the elevated part of the nasal crest.

Independent origin of the infravomerine process has been observed in the sheep, pig and man by Biondi (1888) and in man by Fawcett ('11).

From my own studies it seems clear that the premaxilla consists of a body which holds the two incisors and which is covered by a bony plate of the maxilla proper. It is composed of two processes. One process is directed upward and contributes to the formation of the frontal process of the completed maxilla and the other, or palatine process, grows backward to lie under and support the anterior, pointed extremity of the vomer. The infravomerine process united with the medial part of the palatine process forms the anterior wall of the incisive foramen.

The maxilla proper arises in the membranous tissue which invests the external surface of the cartilaginous nasal capsule. It first appears at the end of the 6th week as a small triangular lamella antero-medial to the ocular vesicle in a position above the canine tooth germ. Rapid growth of the bony lamella takes place in various directions. In the 7th week it extends along the external surface of the nasal capsule antero-superiorly to form the frontal process and downward to form the outer alveolar wall and at the same time it grows forward and backward. A little later it radiates postero-medially to form the palatine process and downward and inward to form the internal alveolar wall. In the 8th week the frontal process reaches a height almost comparable to its whole length and the bony lamella unites with and covers a part of the premaxilla anteriorly. At the same stage, the bone grows backward inferior to and at some distance from the infra-orbital nerve. The growing bone splits in the region of the origin of the anterior superior alveolar branch of the infra-orbital nerve affording passage for the nerve through it on its way to the incisor teeth. The two extremities reunite later posterior to the anterior superior alveolar nerve. The outer limb of the fork forms the zygomatic process, the inner one, the orbital plate of the maxilla. At the latero-posterior portion of the frontal process the bone splits form-

ing the anterior part of the infra-orbital canal. The lateral extremity grows above the infra-orbital foramen and meets the medial extremity, but the two extremities may remain separated as the infra-orbital fissure.

During the 9th week a bony lamella radiates medio-posteriorly from the maxillary center above the canine tooth, the perpendicular portion of which forms the medial wall of the maxillary body and the horizontal portion of which unites medially with the palatine process but remains separate from it laterally.

At the 10th week the zygomatic process attains a great size and is separated from the outer alveolar wall by a relatively wide bar of bone.

At the end of the third month the palatine portion has greatly thickened. The frontal process has by this time usually lost the bifurcation on its facial aspect (fig. 8). The perpendicular part of the bony lamella radiating from the maxillary center forms the medial wall of the body of the maxilla. There is a small space or groove existing between the medial wall of the body of the maxilla and the internal alveolar border. This is the beginning of the maxillary sinus. It undergoes only slight change in shape and size during the next fetal months. At about two years after birth when the permanent teeth erupt it begins to increase greatly in size. Eventually, by pushing outward between the floor of the orbit and the roof of the tooth sockets, the maxillary sinus attains its full size.

From the three month fetal stage onward there is little of note to be recorded excepting a general enlargement of the bone in all directions. However, there is one marked difference between the fetal maxilla and the adult bone, i.e., the poor development of the height of the former. The shallowness of the young bone is striking. In the 4 month old fetal skull the height of the maxilla from the lower border of the alveolar process to the orbital plate is only about 5 mm, and the breadth from the median palatine line to the external alveolar border is about 7 mm. At this stage, the lower mar-

gin of the alveolar border is about at the same level as the palatine process. There is no great increase in height until about two years of age when the eruption of the deciduous teeth is completed and thereafter the height of the maxilla exceeds its breadth.

Figures 13 and 14 show the oral and nasal views of the maxilla and palate bone at the age of 4 fetal months; figures 15 and 16, at the age of 8½ fetal months (note the premaxillary sutures on both the oral and nasal surfaces).

One of the interesting variations in the palatine portion of the maxilla is the medio-palatine bones (Woo, '48b). Figure 1 shows complete medio-palatine bones in a fetus of about 8 months of age. It is interesting to note that the outline of the bones does not show a trace on the nasal or superior surface. It seems to indicate that only the inferior compact bony layer of the palatine processes of the maxillae is involved in the formation of the medio-palatine bones.

The term maxilla as is now generally used in human anatomy including the premaxilla is confusing and misleading. The description of this part in textbooks of human anatomy is inadequate. In Morris' Anatomy ('42), it is stated: "Running laterally from the incisive fossa to the space between the second incisor and canine tooth, an indistinct suture may sometimes be seen, indicating the line of junction of the maxillary and premaxillary portions of the bone" (p. 160). Gray's Anatomy ('48) states: "On the under surface of the palatine process, a delicate linear suture, well seen in young skulls, may sometimes be noticed extending lateralward and forward on either side from the incisive foramen to the interval between the lateral incisor and the canine tooth. The small part in front of this suture constitutes the *premaxilla (os incisivum)*, which in most vertebrates forms an independent bone" (p. 167). The premaxilla in man as stated above has definite form and boundaries and is an equivalent structure to the premaxilla of other mammals. For the sake

of showing the phylogenetic relations, it is suggested that the term maxilla should not include the premaxillary bone and the term premaxilla be retained in man.

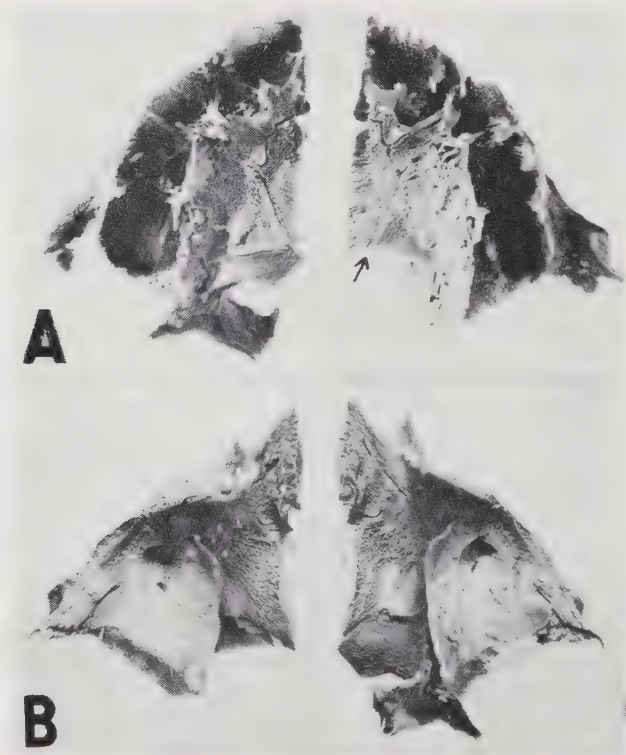


Fig. 1 Fetal palate of 8 months with complete medio-palatine bones showing on the oral surface. Note that not a trace of the outline of the bones shows on the nasal surface. A, Oral (upper) and B, nasal (lower) views. $\times 2$. (Courtesy of Dr. T. D. Stewart.)

GROWTH OF THE PALATE BONE

Ossification of the palate bone commences in membrane at the end of the 7th week at the junction of the vertical and horizontal portions. It first appears in the form of a tiny crescent placed antero-posteriorly with its concave surface facing the medial side. The tiny bony piece extends rapidly upward to

form the vertical plate and medialward to form the horizontal plate. During the 8th week it spreads backward to form the pyramidal process and downward to form the tuberosity. At the 9th week the two branches of the pyramidal process are seen to be widely separated for the internal pterygoid plate (fig. 6). The vertical plate at this time is seen to curve medialward almost by 45° though at later stages it comes to lie at much more of a right angle to the horizontal plate.

In the 10th week the sphenoidal and orbital processes begin to develop as outgrowths from the vertical plate. At the junction of the vertical and horizontal plates the bone extends forward and begins to overlap the maxillary bone (fig. 9). The vertical plate is fan-shaped and the horizontal one is of a quadrilateral form, the medial side of which is about three-fifths of the lateral side.

When the medial wall of the body of the maxilla is formed at about the end of the third month, a small portion of the lateral part of the horizontal plate of the palate bone is actually inserted between the layers of the palatine process of the maxilla. The anterior portion of the vertical plate overlaps almost one-third of the medial wall of the body of the maxilla with the part of the palate bone at the lateral side. This portion of the vertical plate of the palate bone forms approximately one-half of the medial wall of the maxillary sinus. The anterior portion of the palate bone which overlaps the maxillary bone has been neglected in textbooks of human anatomy, and the vertical plate is erroneously described as oblong in shape. The sphenoidal and orbital processes extend at this stage more upward and grow larger. The sphenoidal process is always larger than the orbital one throughout fetal life.

The measurement of the horizontal portion is greater than the vertical throughout fetal life. At birth the two parts are about equal in length. After birth the vertical plate exceeds the horizontal one and the orbital process outgrows the sphenoidal.

SUMMARY

Eighty-four human embryos and fetuses ranging in age from 5 weeks to full term were cleared and stained. In addition, 34 skulls ranging in age from birth to maturity and 319 sagittally sectioned adult skulls were observed.

The maxilla proper is developed from one ossification center beginning at the end of the 6th week. The premaxilla is ossified from two centers; the main center arises at the middle of the 7th week and the infravomerine center at the end of the 10th week. One ossification center appearing at the end of the 7th week gives rise to the palate bone.

The maxillary center forms the frontal process and the outer alveolar wall in the 7th week and in the 8th week extends forward to embrace the premaxilla on the facial aspect (however, the separation of the two bones still may be seen on the nasal side at birth and on the oral side in young adults). A little later the palatine process and the internal alveolar wall are formed, and the superior compact bone layer of the palatine process separates from the inferior one and turns upward to form the medial wall of the maxillary sinus. The sinus remains a narrow space until about two years after birth. The fetal maxilla is marked by its shallowness.

The adult premaxilla consists of a body and two processes, the frontal and the palatine. The infravomerine process of the embryo unites with the palatine process as soon as it appears in the 10th week.

In the 8th week, the center of the palate bone spreads rapidly to form the vertical and horizontal plates and a little later, the pyramidal process and the tuberosity. The two branches of the pyramidal process are at first widely separated for the internal pterygoid plate. The sphenoidal and orbital processes begin to develop at the 10th week. The vertical plate is fan-shaped instead of oblong as is generally described. A small portion of the lateral part of the hori-

zontal plate of the palate bone is inserted between the layers of the palatine process of the maxilla. The anterior portion of the vertical plate of the palate bone overlaps the medial wall of the body of the maxilla with the former at the lateral side. The length of the horizontal exceeds that of the vertical plate until after birth.

The writer is indebted to Dr. Mildred Trotter for direction and help and to Dr. John C. Finerty for reading the manuscript.

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PLATE 1

EXPLANATION OF FIGURES

Human embryos and fetuses showing the beginning of the appearance of the ossification centers of the premaxilla, maxilla and palate bone.

2 From an embryo of 18 mm C-R length, estimated to be 48 days, showing the beginning of the appearance of the ossification center of the maxilla. Dark ovoid areas are eyes. Anterior view. $\times 8$.

3 From an embryo of 21 mm C-R length, estimated to be 51 days, showing the beginning of the appearance of the main premaxillary ossification center. Supero-anterior view. $\times 8$.

4 From an embryo of 27 mm C-R length, estimated to be 55 days, showing the beginning of the appearance of the ossification center of the palate bone. Inferior view. $\times 8$.

5 From an embryo of 62 mm C-R length, estimated to be 75 days, showing the beginning of the appearance of the accessory (infravomerine) premaxillary ossification center. Antero-inferior view. $\times 8$.

Mx., maxilla; Pm., premaxilla; Man., mandible; Cl., clavicle; Pl., palate bone; Inf., infravomerine center of premaxilla.

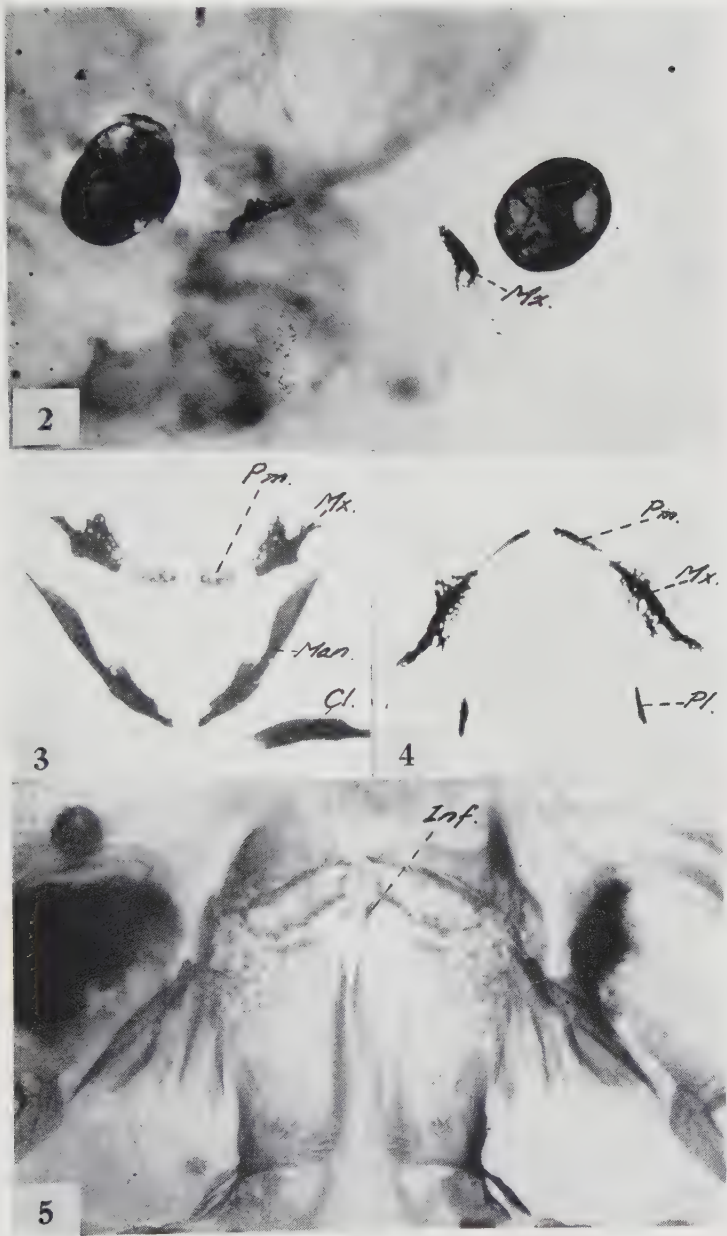


PLATE 2

EXPLANATION OF FIGURES

Human embryos and fetuses showing the obliteration of the premaxillary portion on the facial aspect.

6 Antero-inferior view of the upper jaw of an embryo of $8\frac{1}{2}$ weeks, showing the separation of the maxilla and premaxilla. $\times 8$.

7 Anterior view of the upper jaw of an embryo of 9 weeks, showing the separation of the maxilla and premaxilla. $\times 8$.

8 Lateral view of head of a fetus of three months showing the covering of the frontal process of the premaxilla by that of the maxilla. $\times 8$.

Pm., premaxilla; Mx., maxilla; Pl., palate bone; Pt., pterygoid plate.



PLATE 3

EXPLANATION OF FIGURES

Human embryos and fetuses showing the palatine process of the premaxilla.

9 Inferior view of the palate of an embryo of 10 weeks showing the transverse bony lamellae (arrow) of the palatine processes of the premaxilla. $\times 8$.

10 Inferior view of a fetal palate of three months. Note the longitudinal infravomerine process of the premaxilla. $\times 5$.

11 Inferior view of a fetal palate of three and one-half months. Note the longitudinal infravomerine process of the premaxilla. $\times 5$.

12 Incisive (inc.) and interincisive (int.) sutures in an American Negro male skull, age 30 years.

Mx., maxilla; Vo., vomer; Pl., palate bone; Pt., pterygoid plate; Inf., infravomerine process of the premaxilla.

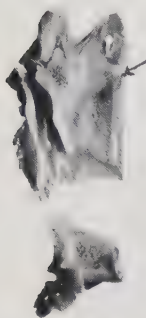


PLATE 4

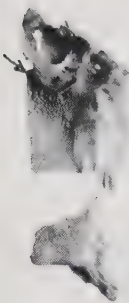
EXPLANATION OF FIGURES

13 and 14 show the nasal and oral surfaces, respectively, of the maxilla and palate bone at 4 fetal months. Note the premaxillary sutures on both surfaces. $\times 2$.

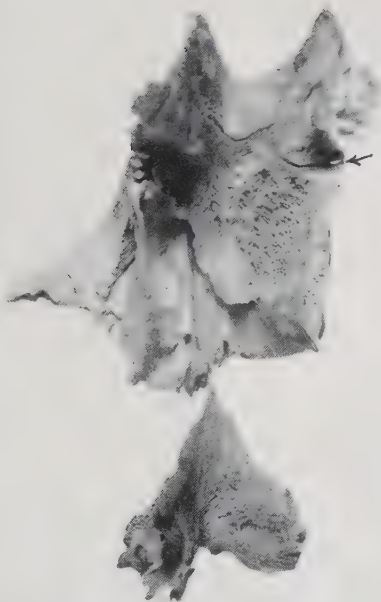
15 and 16 show the nasal and oral surfaces, respectively, of the maxilla and palate bone at $8\frac{1}{2}$ fetal months. Note the premaxillary sutures on both surfaces. $\times 2$.



13



14



15



16

BREADTH OF EPIDERMAL RIDGES IN THE HUMAN FETUS AND ITS RELATION TO THE GROWTH OF THE HAND AND FOOT¹

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SIX FIGURES

INTRODUCTION

This study was undertaken to determine: (1) the growth of epidermal ridges during the prenatal period; (2) the history of the details (minutiae) of individual ridges with particular reference to the question of whether new minutiae appear as the ridge systems develop.

The development of epidermal ridges, as regards topography, has been studied by Kölliker (1848-1849), Bonnevie ('27, '29, '32), Schaeuble ('33), and Gould ('48). The findings of Gould may be summarized for orientation of the present work.

Epidermal ridges first make their appearance on the apical pads of the fingers at 13 weeks menstrual age (68 to 70 mm C.R.). Ridges next begin to develop on the apical pads of the toes. The distal regions of the palm and sole become ridged shortly after the apical pads of the respective members. The earliest of these ridges occur in relation to the interdigital pads, located at the level of the metacarpophalangeal and the metatarsophalangeal joints. The development of

¹From a thesis submitted (on May 2, 1949) to the Department of Anatomy of the Graduate School of Tulane University in partial fulfillment of the requirements for the degree of Master of Science.

ridges on the proximal regions of the palm and sole follows, thus completing the extension of ridged skin.

Work of a quantitative nature concerning the epidermal ridges in the fetus is scanty. Measurements of ridge breadth in younger fetuses have been totally lacking. Hecht ('24) measured the breadths of ridges in the finger patterns of three premature infants, 7 newborn infants, and in children. Cummins et al. ('41) and Ohler and Cummins ('42, '45) studied in detail the breadths of epidermal ridges in young adults.

Evidence that the definitive configurational type of a ridge system is laid down at the time of differentiation is supplied by the work of Kölliker, Bonnevie, Gould, Cummins and Sicomo ('23) and Cummins ('23, '26). The prime object of the present work was to determine whether the configuration as seen in the later prenatal period differs from its original state only in the increased ridge breadth or also in features introduced by ridge multiplication.

The author wishes to express his indebtedness to Dr. Harold Cummins who suggested this study, and whose guidance has materially aided it, to Drs. Ralph N. Baillif and Theodore Snook, for advice on technical procedure, and to Dr. George W. Corner for the hands and feet of 10 fetuses (70–110 mm) from the Carnegie Collection.

MATERIALS AND METHODS

The material used in this study (table 1) consists of 129 hands and 132 feet of a graded series of fetuses ranging in size from 70 mm C.R. to 350 mm C.R.; the menstrual ages range from 13 to 40 weeks according to the usual standards (Mall, '18; Streeter, '20; Scammon and Calkins, '23; Arey, '25). These specimens, all of which had been preserved in formalin, were prepared in the following manner.

The hands and feet were amputated and then washed in running water for 24 hours. The cornified epithelial cells

were rubbed away with a soft gauze pad, a procedure that was found necessary because the impermeability of these superficial layers hindered staining of the epidermis.

A large flat dish was filled with clean sand, to which Harris' hematoxylin solution had been added. The sand served to support the specimen at the desired level in the stain. Best results were obtained when the stain reached the level at which the volar surface only of the specimen lay in a puddle of stain. The optimum staining period varied with the size of the specimen; the specimens were removed when the volar

TABLE 1

Distribution of material (stained and cleared preparations excepting 5 fetuses in the last two groups for which it was necessary to make prints instead).

C.R. LENGTH		NO. HANDS	NO. FEET
Range	Average		
<i>mm</i>	<i>mm</i>		
70- 90	77.6	8	9
90-110	96.6	13	13
110-130	115.0	12	13
130-150	136.9	24	23
150-170	155.0	20	19
170-200	179.0	21	23
200-250	210.0	13	14
250-350	314.3	18	18

surface acquired an even purple color (usually about 5 minutes). After staining, the hands and feet were dehydrated with dioxane, then cleared and stored in oil of wintergreen. The general appearance of a specimen prepared by this method is illustrated in figure 1.

The amount of shrinkage induced by this procedure was determined in a small series of specimens. Length and breadth measurements were taken on hands and feet before and after the stage of dehydration and clearing. It was found that dioxane caused less shrinkage than ethyl or butyl alcohol. In the larger specimens (200 mm C.R. and upward) the shrinkage did not exceed 2 mm in length and 1 mm in

breadth, or less than 5% of either measurement. In the smaller size groups, the shrinkage was not detectable by the method used for the measurement of length, to be described later.

Only specimens showing the dermatoglyphic features clearly were used in this study. It was found difficult to secure properly fixed specimens of the final size group (300 mm C.R.) and the prints of 5 stillborn infants were added to the material representing this group.



Fig. 1 Plantar surface of a 120-mm fetus, photographed to show the appearance of the stained and cleared preparations used in this study. $\times 11$.

The hands and feet were grouped according to the C.R. lengths of the fetuses from which they had been taken (table 1). The indicated measurements of hands and feet are those taken after dehydration and clearing.

OBSERVATIONS

Growth of the hand and foot

Hand length and foot length were measured according to the conventions suggested by Schultz ('29), with the use of the specially constructed device described below. For the hand, the measurement was taken from the carpale to the tip of the third finger and for the foot, from the pternion to the tip of the second toe. Specimens were placed on a piece of ground glass upon which two lines were ruled, crossing at right angles. The anatomical axis of the part being measured was aligned with one ruling in a position such that the most proximal measuring point (carpale or pternion) was just enclosed by the other ruled line when viewed from above. A vernier, with its zero mark set at the second ruling, was then adjusted until the distal point of measurement became enclosed.

Studies by Schultz ('24, '26), Midlo ('34), Midlo and Cummins ('42), and Köenner ('38) are concerned mainly with the ontogeny and phylogeny of the primate hand. Measurements of the hands of children, young adults and adults are numerous in the literature; however, information concerning the growth of the human hand during the fetal period is scanty.

It has been observed, from the measurements of the fetuses used in this study, that the growth of the hand is linear. This linearity can be explained on the basis that the series of fetuses studied represents only a short straight-line segment of the entire growth period. Figure 2 illustrates the linear nature of the growth of the fetal hand and it is of note that a large component of the linearity is due to the nature of

the plot. That is to say, both hand length and C.R. length vary exponentially with respect to time.

Measurements describing the growth of the foot in man and other primates are abundant. Mall ('18), Streeter ('20), Seammon and Calkins ('23), Schultz ('24, '26), Straus ('26, '27), Davenport ('32), and Meredith ('44), have all made valuable contributions to our knowledge of this subject. Streeter ('20) has assembled a comprehensive array of measurements on the growth of the human foot during the fetal period. The present data closely approximate those of

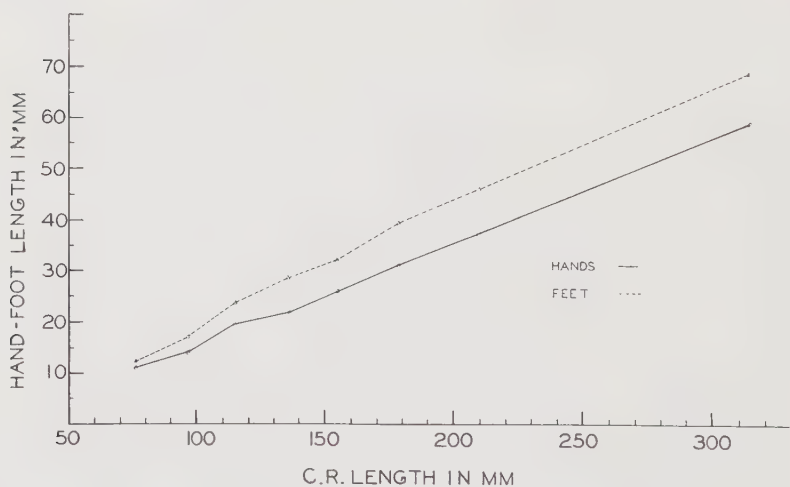


Fig. 2 Growth in length of fetal hands and feet.

Streeter, except for a discrepancy in the larger size groups. The author's measurements give higher values for foot length in fetuses larger than 200 mm C.R. It is possible that the difference arises from the unlike racial composition of the two series, though it may be attributed also to the small size of the sample. Streeter's material was largely composed of white fetuses, while this series contains a larger proportion of negro fetuses.

The growth curve of the foot (fig. 2) is, for the period studied, similar to that of the hand. The rate of growth (the slope) is approximately equal in the two members. If these

curves were extrapolated back into the earlier period, they would cross each other in accordance with the rule of cranio-caudal differential growth.

Ridge breadth

No information has been available concerning the increase in breadth of epidermal ridges during the fetal period. Before presenting the findings, the areas measured are to be identified and the method of measurement described.

Cummins ('29) has described the history of the volar pads in the fetus. These pads are the sites on which localized ridge systems develop. Further, Cummins ('26, '41) and Cummins and Sicomio ('23) have suggested the importance of differential growth as a factor in the conditioning of ridge direction. It follows that the areas in which the ridges are measured are of the utmost importance, since the various regions of the palm and sole are subject to varying rates of growth. It is also desirable that several regions in the palm and sole be measured in order that a representative average be obtained. The areas measured should have approximately the same rates of growth; for this reason the thenar, hypothenar, interdigital I and interdigital IV areas have been chosen. The areas measured in hand and foot are homologous.

Ridge breadth was measured with a filar ocular micrometer substituted for one ocular of a binocular dissecting microscope. The micrometer was set at zero and so aligned that the cross hair was parallel with the ridges in the field. The drum was then rotated until 10 ridges had been traversed by the hair. The distance occupied by 10 successive ridges constitutes the measurement, from which the average ridge breadth for that one determination is derived.

Three measurements were taken in each of the 4 selected regions of the palm. The average for the specimen consists of the grand average of all the regional averages. The same procedure was followed in the three selected regions of the foot.

Figure 3 records the findings in respect to the growth in breadth of the epidermal ridge. It is to be noticed that this growth is linear with respect to C.R. length. Since the growth of the hand and foot was found to be linear in nature, the high coefficient of correlation ($.83 \pm .01$ in the hand; $.70 \pm .03$ in the foot) between ridge breadth and hand and foot length was to have been expected.

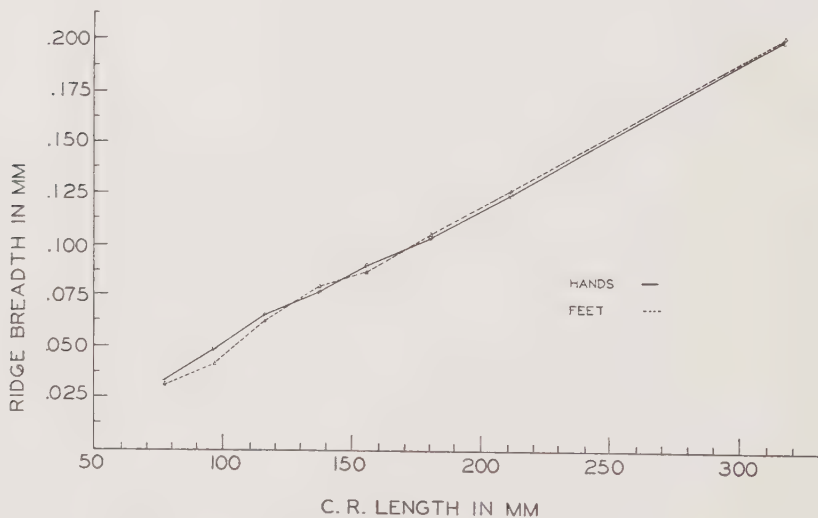


Fig. 3 Growth in breadth of epidermal ridges.

Number of minutiae

The purpose of determining the number of minutiae is to test the ability of the developing ridge to multiply. The term minutiae refers to the details of the morphology of a single ridge, including such features as branchings, interruptions in the continuity of a ridge, and the isolation of short ridge units.

The number of minutiae per standard area was used as the unit of comparison. It is evident that if a uniform area were used as a measure in all size groups, the relative area measured would decrease in specimens of larger size. In order that the relative areas measured be kept constant, they

must be proportioned to the varying absolute areas. Also, since the relative growth rates of the hand and foot differ, separate measures for each are necessary. This was accomplished by plotting the growth of the hand and foot on rectangular coordinate paper, and then calculating the slope of the line of best fit. The slope was then used to calculate the size of area needed for each size group. The appropriate area was drawn on a piece of ground glass and the specimen projected at standard magnification ($\times 3$).

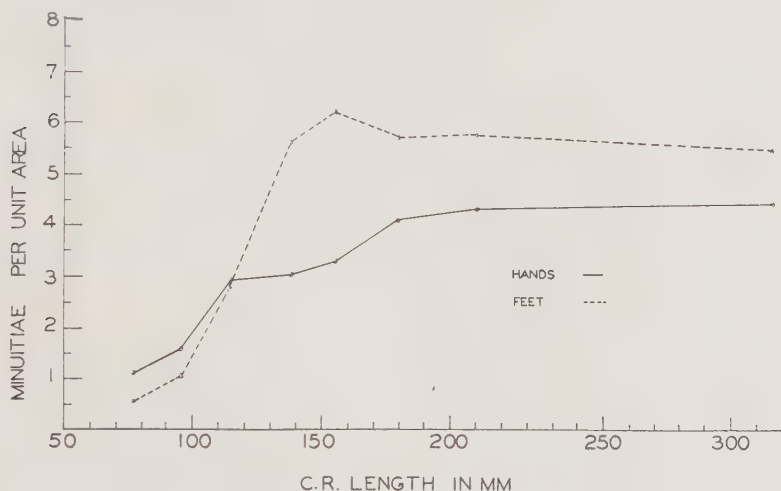


Fig. 4 Number of minutiae per standard area.

Figure 4 represents a plot of the average number of minutiae per standard area for each size group. These averages were derived in the following manner. The minutiae enclosed by the appropriate square were counted in each of the areas previously described for the hand and foot. The individual average represents the grand average of three such measurements. The values for the size groups are averages of all the individuals within each group.

It was found that there is an increase in the number of minutiae per standard area (fig. 4). Of particular interest is the fact that at 115 mm C.R. length the curves for the counts of minutiae in hand and foot cross one another. It was pointed

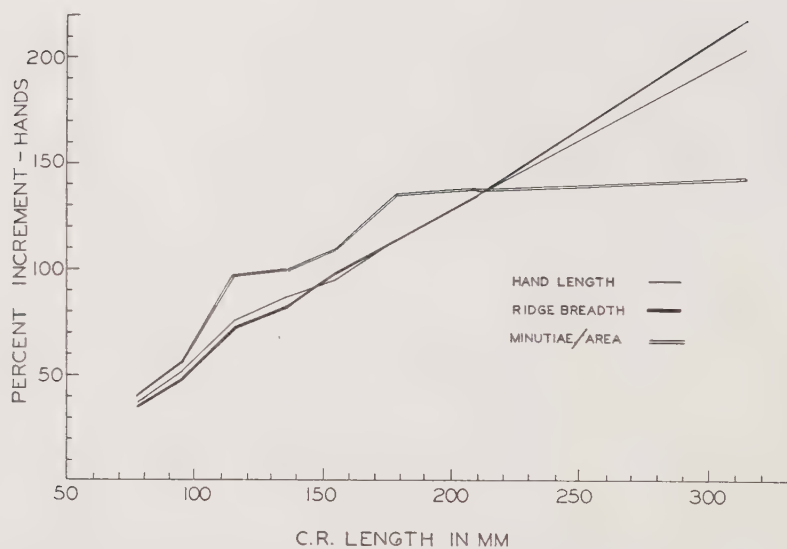


Fig. 5 Percent increments of hands: hand length, ridge breadth, number of minutiae per standard area.

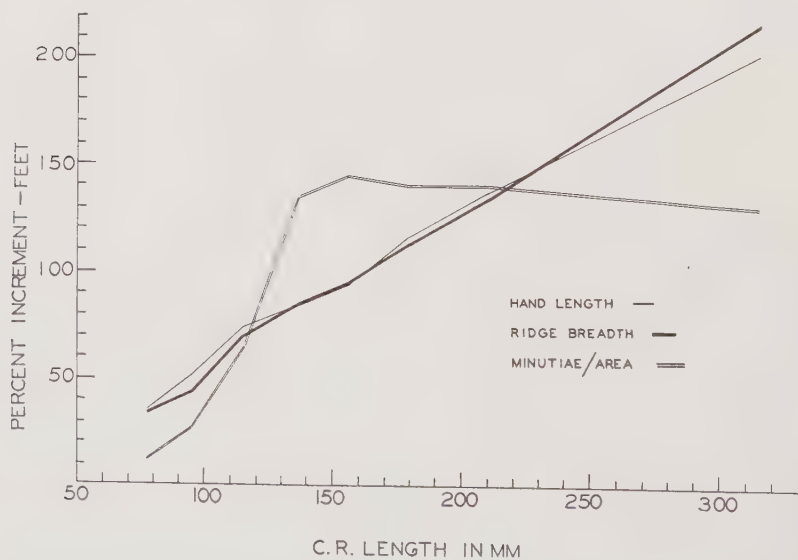


Fig. 6 Percent increments of feet: foot length, ridge breadth, number of minutiae per standard area.

out earlier that the curves for length of hand and foot would cross in a similar manner if they were extrapolated backward in time. The intersection of these curves for minutiae per area illustrates the craniocaudal differential growth principle. Of note also is the fact that the rate of multiplication of ridges in the foot, as evidenced by the marked increase in minutiae, between 100 and 150 mm C.R. length, demonstrates a more rapid rate of differentiation in that part.

The deflection point of both curves (approximately at 140 mm C.R. length) is significant, especially in the light of percent increment analyses (figs. 5 and 6). These analyses show a tendency in growth for ridge breadth to lag behind hand and foot length. At 140 mm C.R. length this condition reverses and ridge breadth increments are equal to or exceed corresponding increments in length. It is significant that during this critical period, 140 mm C.R. length, the increments of minutiae reach a plateau.

DISCUSSION AND CONCLUSIONS

The works of Kölliker (1848-1849), Bonnevie ('27, '29, '32), Schaeuble ('33) and Gould ('48) describe in detail the appearance of ridges on the volar surface. The volar pads serve as foci for the developing ridges, which are seen to extend from these centers in all directions until the entire volar surface is ridged. The ridges first make their appearance when the volar pads have passed their peak in development (and are thus becoming less prominent). Certainly the developing ridges cannot escape the effects of differential growth since they first arise within structures which are greatly influenced by this growth process.

This study is the first to consider quantitatively the developing ridge in the fetus. Hecht ('24) measured the breadths of epidermal ridges in three premature infants, although his study was concerned mainly with growth of the epidermal ridge during the postnatal period. Hecht's findings concerning premature infants fit well within the limits of the author's measurements of fetuses of comparable age.

Cummins ('23, '26) and Cummins and Sicomo ('23) emphasize the importance of differential growth in the conditioning of ridge direction. Cummins, Waits and McQuitty ('41), and Ohler and Cummins ('42, '45) have analyzed regional variations of ridge breadth in young adults. Their findings are of special interest in this work since coefficients of correlation for ridge breadth and hand length (and foot length) are of considerably smaller magnitude than the author's coefficients. In the adult, coefficients of correlation are .30 and .22 for the hand and the foot respectively. During the fetal period the coefficients for the hand are .83, and for the foot .70. The principal factor producing this discrepancy in values is the wide range of sizes in the series of fetal hands and feet. There is a smaller range of variation of adult hands and feet and a correspondingly greater expression of individual differences in the breadths of epidermal ridges.

The percent increment in growth of the hand and foot (figs. 5 and 6) in the fetus is observed to exceed the growth of the ridge in breadth. Since the volar surfaces are continuously ridged, multiplication of the number of ridges must account for the discrepancy in growth rates. Minutiae, defined as irregularities in the continuity of a ridge, reflect the formation of new ridges subsequent to the period of initial differentiation. Figure 4 shows that minutiae increase in number. This tendency of the ridges to multiply is seen to be greatest during the period of maximum difference between the increments of increase in surface length and ridge breadth.

Based upon these data, derived from 129 hands and 132 feet of fetuses ranging from about 13 weeks to term, the growth of the epidermal ridge in breadth may be described as progressing through a series of three stages.

Ridges are first laid down at approximately 13 weeks. It is during this period that the general configuration of the ridged skin is determined, and increase in breadth of the ridges accompanies growth of the volar surface.

After ridges have covered the volar surface, their increase in breadth does not keep pace with the growth of that sur-

face. It is during this period (14–19 weeks) that the ridges undergo multiplication, new ridges being inserted within the general configuration. Multiplication continues until enough ridges have been added to the system (19 weeks) to allow the increase in the breadth of the ridges to keep pace with growth of the surface, and the increase of minutiae is incidental to this multiplication. When ridge multiplication ceases the definitive configuration is established in all details.

The third phase (from 19 weeks onward) is one of stability. That is to say, no new ridges develop and as the part grows the ridges only broaden and lengthen.

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ERRATUM

An error occurs in the authors names as printed in Abstract No. 286, page 615 of The Anatomical Record, volume 105, no. 3 and page 135 of the ASZ reprints. The title is reprinted below as it should appear.

286. A PERSISTENT RHYTHMICITY IN UCA RED CHROMATOPHORES. Frank A. Brown, Jr., H. Marguerite Webb, and J. Bruce Guyselman; Cresap Biological Laboratory, Northwestern University, and Marine Biological Laboratory, Woods Hole, Mass.

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